

Your man in Whitehall

TWICE in the past three months the absence of a Chief Scientific Adviser to the government has been pointedly remarked on. Sir Alan Hodgkin, retiring President of the Royal Society, drew attention to the vacant post in his farewell address in November. Now the Commons Select Committee on Science and Technology, in an exchange of letters with the Prime Minister, brings the issue to the surface again. But are those who ask for one person to head government's central machinery for co-ordination of policymaking really clear that the system would thereby work any better than it does at present?

The post of Chief Scientific Adviser based in the Cabinet Office has been vacant for almost two years. Sir Alan Cottrell, having served for his five-year term, returned to academic life. His deputy, Dr Robert Press, a career civil servant with a background in nuclear matters, took over responsibility for science and technology without being named as chief adviser. Dr Press retires this summer; hence the rumblings.

The Select Committee says that in the past its suggestion of a ministry for research and development was brushed aside because the Chief Scientific Adviser was already said to be responsible for ensuring adequate co-operation and co-ordination between departments. In the absence of such an adviser, the committee says, "[we] wonder whether assurances given by successive governments about the co-ordination of research and development continue to represent government policy . . . failure to fill the vacancy has caused considerable dismay in the scientific community, and has raised doubts about the sincerity of the government's commitment to the importance of an independent scientific voice in the formulation of policies at the highest level."

In reply, the Prime Minister emphasises that Dr Press's appointment was intended as transitional, but that he has in mind stronger central co-ordination—though the appointment of Chief Scientists in many ministries "emphasised the increasing importance attached to scientific advice and underlined the impracticability of providing this from one central point. The creation of the Central Policy Review Staff as a multidisciplinary body of advice to the Cabinet also added a new dimension." Mr Wilson missed an excellent opportunity very recently of further sounding out views on science in government by devoting most of a speech to the Parliamentary and Scientific Committee to fulsome praise for the National Research Development Corporation (repeated almost verbatim from the Blackett Memorial Lecture of three months earlier), and by relegating questions of science in government to a few sentences on the

difficulty of pleasing everyone all the time. The question of advisers in general was passed off with a joke about an advisory bull belonging to FAO.

The real trouble with the post of Chief Scientific Adviser is that he is placed in such an ambiguous position. The Select Committee's letter refers to at least four functions:

- responsibility for interdepartmental coordination and co-operation
- co-ordination of research and development
- an independent scientific voice in policy formulation
- articulation of scientific advice to the Cabinet.

The academic community would undoubtedly add a fifth—provision of a direct channel for academic scientists to have access to government particularly when they held views which diverged from those expressed by civil service scientists. Industrial scientists might add further functions. The job begins to look attractive only to a megalomaniac or a superman.

Further, neither of the last two advisers exactly raved about the job after their departure; they certainly gave the impression that they found it rather frustrating. If famous names were now to be canvassed for the post, the government would have a lot of arm-twisting to do. And if the scientific community has been expressing dismay at the lack of a Chief Scientific Adviser, it has been doing so in a most *sotto voce* way.

Mr Wilson declares that he wants stronger central co-ordination but can do without the centralised advice, so he will probably look for a sound scientific administrator from within the civil service rather than a famous name from without. If he views the civil service as the sole source of scientific input to the government, this makes sense. But hundreds of millions of pounds are spent each year on nurturing science and technology both in academe and industry. Much of this expenditure will not result in anything that the government desperately needs to know about, but the existence within the Cabinet Office of some sort of external liaison office accessible to research councils, industrial organisations and individual scientists might well ensure that when matters of importance do turn up, they do not get tangled up in Whitehall bureaucracy and interdepartmental rivalry.

The post of Chief Scientific Adviser should no longer be retained. But when Mr Wilson recruits his top co-ordinator for departmental science he should also hire a good scientist from outside the civil service as liaison officer with the outside world.



Change and consequence

In a second concluding article, **Peter Pockley** looks at Australian science and the recent election.

SCIENCE was not a starter in the public campaigning for the December election although there was plenty of activity behind the scenes. The Liberal Party did, however, make three mentions of science which were important in the light of subsequent events. This made the score Liberals 3, Labour 0, for the Labour campaign concentrated on dramatising the constitutional issue of the powers of the Senate and the essentially political actions of the Governor General in bringing down the popularly elected government.

The first mention came in Mr Fraser's policy speech, in which, as an item of evidence for ineptness in the Labour administration, he took a passing swipe at the research grants controversy. The second item was the Liberal Party's issuing of a statement on science policy in which the Department of Science retained a central role. Mr Fraser's acceptance of *any* science policy is a political turn-around. In 1968, as Minister for Education and Science, he had argued in a major speech before an Academy of Science meeting that attempts to define a science policy were futile. This *volte face* paled into insignificance, though, before some others which Mr Fraser underwent but which he successfully played down during the campaign: he had, for instance, been one of the strongest advocates and administrators of compulsory military service and of the ultimately futile expedition by Australian forces, including conscripts, to the Vietnam war, policies which he could not afford to embrace in 1975.

Mr Fraser got away with such inconsistencies by developing a form of non-speak which he used successfully to deflect all specific questions with varying combinations of a few generalised, qualified or conditional phrases. This technique, which tied him down to almost no action of detail or permanent substance, was aggravating in the extreme to working journalists and political opponents alike, but such was the strength of feeling he generated against Labour that he had no cause for deigning to answer questions with facts or commitments. An example of this approach was provided by the third mention of science in the campaign.

Only five days before the election, Mr Fraser personally issued a statement designed to quell speculation that the Australian Science and Technology Council (ASTEC) would be abandoned or greatly modified by a Liberal government which had arisen from some

remarks on a radio programme by the then Liberal spokesman on science, Mr Eric Robinson. The statement largely affirmed the objectives defined for ASTEC by Labour (although not by name, of course) and went on to say that it will report directly to the Prime Minister. This plan was welcomed by many scientists who had not been happy with Labour's arrangement in which ASTEC reported to the Minister for Science and Consumer Affairs. This much, at least, of Mr Fraser's statement calmed troubled spirits who felt even more secure from the statement that ASTEC "will be a body of the highest status and greatest independence".

It seems to have been only after the election that observers realised that the statement quoted gave no guarantee of independence, except in the way that that term may be defined by a conservative government. Labour was guaranteeing independence for ASTEC by making it a statutory corporation operating under its own Act of Parliament and requiring it to publish its findings. The necessary legislation was, however, one of many important Labour Bills already under debate but which were nullified by the proroguing of Parliament. ASTEC has, in fact, been operating only on an officially "interim" basis.

What happens to ASTEC now is a matter of guesswork. The small organisation has been transferred to the responsibility of the Prime Minister. The Chairman of the Interim body, Dr J. A. L. Matheson, has left his job as Vice-Chancellor of Monash University in anticipation of early confirmation as full-time Chairman. At the time of writing, if the government is acting on the matter at all, it is doing so with sealed lips. The longer the delay, the greater the speculation that "greatest independence" may mean becoming a box in the organisational chart of the Prime Minister's Department or the Department of Science and being treated as a kind of bureau.

Science department curtailed

No group in the public service would probably be happier with a diminution of ASTEC's independence than the Department of Science, which had never been enthusiastic about ASTEC as a rival in the advisory stakes to government. The department, however, lost much and gained nothing in terms of responsibilities after the election. For a few brief months of potential glory in 1975, the department had

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James Webster (top) and Clyde Cameron

taken on an extra function of considerable political importance. For some time previously, Mr William Morrison had paved the way for his department to fly the popular flag of consumerism. On taking over Mr Morrison's portfolio, Mr Clyde Cameron was presented with the grander title of Minister for Science and Consumer Affairs. Mr Cameron made a rather ungainly start; his set-piece speeches cast grapes at many targets and displayed little sympathy with his new responsibilities. But, to his credit, he soon settled down to becoming a convinced champion of science *per se*, which Mr Morrison had never done.

In the consumer field, he secured a promise of services from Mr Ralph Nader. Substantial legislative powers were being developed by the new Consumer Protection Division of the department which had been given responsibility of the consumer sections of the forceful Trade Practices Act. In a trade-off with the Attorney General's Department which had thereby lost part of the administration of this Act, the Science Department handed over its Patents operations. Earlier in 1975, the Science Department had established a Consumer Standards Branch. In Mr Cameron's short reign, then, his department enjoyed both a monitoring and prosecuting function in the interests of consumers. This useful union

of powers never enjoyed active consummation; if it had, there is little doubt that it was a potential vote-winner for Labour.

In the December shuffle of responsibilities by Mr Fraser, all consumer activities were removed from Science and split between Attorney General's (consumer protection) and a new Department of Business and Consumer Affairs (consumer standards) where it will require devilish determination to prevent a conflict of interest between business and consumers, to the likely detriment of the latter. Patents, incidentally, continue to bounce around; their administration has now gone from Attorney General's to Business and Consumer Affairs. The fate of consumerism under the Liberals mirrors that of environmentalism which has similarly been buried within a large department of diverse interests, namely Environment, Housing and Community Affairs.

Also after the election, the Department of Minerals and Energy was renamed National Resources under the National Country Party Leader and Deputy Prime Minister, Mr Doug Anthony, as Minister. Professor Messel and Sir Lenox Hewitt retain influence in the department's area of responsibility through the Australian Atomic Energy Commission (AAEC), of which Sir Lenox is Vice-Chairman. Two of the five places on the AAEC have been vacant for some time. Mr Connor was probably keeping his empire-building options open; one of these options was thought to be the creation of a new multi-energy research and development authority which would have incorporated the AAEC, the Bureau of Mineral Resources and the Mineral Research Laboratories of CSIRO, all of which were then under Mr Connor's control. Mr Anthony has not held on to the CSIRO bit, and has shown no indication of how he will treat the AAEC.

The disciplined control on information within the Fraser ministry meant that it was not until some time after the election that the most senior people in CSIRO could obtain clarification of the line of responsibility of their organisation. The new Science Minister, Senator Webster, is now solely responsible for the Science and Industry Act which gives CSIRO its statutory independence. The Minister-CSIRO line is direct and separate from the Minister-Department line. In the face of diminished duties, the Science Department is not out of the woods yet; it will have to work hard to maintain its position which could be called into question whenever Mr Fraser reshuffles his Cabinet or when its Secretary reaches retiring age within a couple of years.

Senator Webster himself is somewhat of an unknown quantity. Before his elevation to the ministry, he was known only to the public through having survived a legal challenge to his eligibility to sit in Parliament on grounds of alleged improprieties in dealings between a family company and the government. Since coming to office, Senator Webster has made only the odd routine announcement on behalf of the organisations under him. He is not yet known for having a personal interest in science. However green a minister he may appear at the moment to senior Australian scientists, he is no different in pre-knowledge of scientific affairs from either of his Labour predecessors, and given his parliamentary reputation as a diligent worker on committees, Senator Webster should make something of his job in a stable political environment.

Labour's heritage

If nothing else, the political awakening

of Australia under Labour has produced a quality of information and debate about science policy which is likely to work to the benefit of Australian science in the long run. On a more universal plane, the thoughtful, informed and voluminous reports issued recently, and the subsequent arguments about the very necessity for a Department of Science in any national government, are worthy of separate analysis outside the election context, for the factors involved for science planning may well prove to have global validity.

On the Australian scene, after five years of almost stand-still operations while awaiting times of enlightenment in science policy, it is sad to reflect that the arrival of these very times has coincided with a period of tight-fisted, inflation-dominated management of the economy. Australian scientists had deserved a better deal from both parties; but perhaps they were simply too nice and soft for too long. □

Ringing the changes

Australian science did not escape the impact of the country's political hiatus, as shown by this summary, covering in order the changes over the period beginning a few days before the election:

- The Science Task Force of the Royal Commission on Australian Government Administration recommended abolition of the Department of Science, and redistribution of its functions among other departments.
- Mr Fraser, the second former Minister for Education and Science in a Liberal Government to become Prime Minister (the first was Mr John Gorton), won an outstanding victory, helped by a long-standing imbalance in size of electorates in favour of the conservative parties—Labour received 43% of votes but gained only 25% of seats.
- The first Labour Minister for Science, Mr Bill Morrison (later Minister for Defence) lost his seat by a handful of votes. The second, and last Labour Minister for Science (and Consumer Affairs), Mr Clyde Cameron, retained his seat and immediately lambasted Mr Whitlam for his leadership (which had led to the demotion of Mr Cameron to the Science portfolio).
- Mr Fraser appointed Senator James Webster, of the National Country Party, as Minister for Science, the portfolio which has the lowest seniority. The Department of Science remained in existence, but without consumer affairs.
- The Department of the Environment, another Labour-initiated de-

partment, was abolished and its staff absorbed and reorganised under the umbrella of the new Department of Environment, Housing and Community Development. This department also embraced the Labour-established Department of Urban and Regional Development. Its Minister is Senator Ivor Greenwood, a hard-line conservative.

- The Secretary of the Department of Science, Sir Hugh Ennor, publicly castigated the Task Force's recommendation that his department be abolished, saying it "lacked credibility" and "questionable logic".
- The Australian Academy of Science was publicly drawn into the debate about the usefulness of the Department of Science through the release of its submission to the Task Force. In appealing with feeling for stability in organisation and financing of science, the Academy concentrated on strengthening the advisory roles to government of the Academy itself and the Australian Science and Technology Council (ASTEC); the Academy mentioned the Department only in passing.
- The CSIRO was returned from a dual responsibility to Labour's Minister for Science and Consumer Affairs and Minister for Minerals and Energy to a single Minister for Science under the Liberals.
- The election by staff of a member of the CSIRO Executive, a mild attempt by Labour at worker participation, was stopped in mid-flight just before nominations closed. Official reason—to give time for re-examination of the implications.

BRITAIN

'Cuts' both ways

The UK Government last week unveiled its White Paper on Public Expenditure and dismayed practically everyone. Colin Norman looks at the implications for British science

GRADUAL erosion of government support for so-called big science, such as high energy physics and astronomy, little real growth in other areas of basic research, and a virtual freeze on university appointments. Those gloomy prospects are held out in the White Paper which sets out tentative plans for public expenditure for the next five years, and it represents a painful re-ordering of some of the Labour government's most cherished domestic priorities; it has provoked howls of protest from various sources, including the resignation of a junior minister.

The basic strategy is a desperate attempt to curb the inexorable growth in public expenditure by cutting back heavily in such sensitive areas as education and social services as well as defence. The 'cuts' themselves, which are only being made in the expenditure *planned* for the next few years and thus aim to prevent the growth in spending which would otherwise have occurred, are designed to have their greatest impact in a couple of year's time: the hope is that their effect on unemployment will be mitigated by the fact that worldwide economic recovery should have filtered through to Britain by then. The proposals should not be taken as Gospel, since they will be reviewed each year and they are unlikely to be put into effect if economic recovery doesn't materialise. But the trends are important, and the dismal state of Britain's economy now seems to make it imperative that some blood will be spilled by the Treasury's knife. For the past couple of years, the government has tried to stave off unemployment by spending heavily on public programmes, with the result that with inflation it has run up a massive national debt, for which it is now forced to make staggering interest payments that could outweigh the savings themselves. Unless the debt and the interest payments can be reduced, politically unacceptable tax increases will be inevitable.

Science and technology

As far as science and technology are concerned, the government is proposing to hold support for basic research constant until the end of the decade. The amount of money flowing to the research councils would increase mar-

ginally this year and next, and thereafter it would decline slightly. Within those totals, however, there is a very deliberate trend away from support of large, capital-intensive programmes, which means that the Science Research Council (SRC) is in for a lean time.

The White Paper itself states that the government intends "to reduce expenditure in the areas of 'big science' (high energy physics, astronomy and space science) supported by the Science Research Council, in order to sustain the other sciences (including applied science) supported by that council, and to enable the Agricultural, Medical, Natural Environment, and Social Science Research Councils to continue to develop programmes based on social need as well as scientific opportunity."

That policy was laid down about a year ago, when the Advisory Board for the Research Councils, an advisory body which recommends how the science pie should be divided among the research councils, suggested that Britain's limited scientific resources should be used to support more people by giving priority to less capital intensive efforts. The result was that SRC's budget was cut by about 2% in 1975, and a similar reduction is expected for each year until 1980.

The axe will fall most heavily on high energy physics, with two accelerators in jeopardy. The first will be the 5 GeV accelerator, known as NINA, which is located at Daresbury, near Manchester. It is due to cease operation at the end of 1977. The other is NIMROD, located at the Rutherford Laboratory, support for which, according to SRC sources, is likely to be reduced although no execution date has been settled.

As for space science, officials in the SRC suggest that British contributions to the European Space Agency will be reduced in the next few years, but the details have yet to be worked out. And the prospect for astronomy is that

there will be no large new capital projects in the next five years.

The other research councils are likely to fare rather better than SRC. The Medical Research Council's funds will grow this year by about 1.7%, those for the Agricultural Research Council are expected to grow by about 2%, and the others are likely to expand in similar fashion.

Additional impact

But there are other proposals in the White Paper which could affect the science budget. Proposed support for higher education has been greatly reduced from previous plans. The target now is for a total student population of some 600,000 in institutions of higher education by 1981; until last week, the target was 640,000. One impact of the reduction, according to the White Paper, will be that "there will be little, if any, scope for increasing total staff numbers after 1976-77." That stagnation in university hiring would follow on equally tight period in academic appointments.

Another dismal aspect of the proposals for education is that "capital expenditure on new buildings, . . . will continue to be severely restricted and more intensive use of available premises will accordingly be required". Failure to commit adequate funds to university facilities is indirectly likely to hurt the research councils. There is some fear that the research councils will find themselves paying for equipment and facilities which would normally come from general higher education support funds. Again, the SRC would suffer most.

In another area earmarked for the Treasury's knife, defence research and development, few details have yet been released. But last week Defence Secretary Mr Roy Mason announced that "we are reviewing the future levels of research and development on defence against chemical and biological warfare carried out at the Porton research establishments, with the object of making significant economies". □

Science Budget expenditure analysed by main forms of research and training support (in £ million)

	ARC	MRC	NERC	SRC	SSRC	Total
Research grants and contracts	1.1	8.3	2.2	19.7	3.0	34.3
Research units	1.2	10.6	0.0	0.0	0.5	12.3
Research Council Establishments	3.5	5.9	12.3	34.7	0.0	56.4
Research Council grant-aided institutes	6.7	0.0	1.3	0.0	0.0	8.0
Postgraduate Awards	0.1	1.9	1.5	10.1	3.9	17.5
International subscriptions	0.0	0.6	0.0	24.7	0.2	25.5
Centrally supported schemes and administration	0.5	1.6	1.9	7.2	1.1	12.3
	13.1	28.9	19.2	96.4	8.7	166.3

Expenditure based on 1975-76 figures, excluding expenditure by the Natural History Museum and the Royal Society (£4.6 million).

EMBO

Transatlantic translation

A unique proposal for regulating genetic research in Europe has been developed. Colin Norman reports

A MECHANISM for controlling potentially hazardous genetic manipulation experiments in Europe will soon be proposed by the European Molecular Biology Organisation (EMBO). A special EMBO committee, consisting of 10 leading European biologists, agreed at a meeting last week that regulations already drafted to control such research in the United States are more than strict enough to protect public health, and a statement expected next week will recommend that they should be implemented in Europe.

Although EMBO is purely an advisory body, with no authority to regulate research, its recommendations will probably carry considerable weight. Its proposals are especially relevant for smaller countries which are unlikely to establish their own mechanisms for controlling genetic manipulation experiments, and, if implemented, they would set up a unique arrangement for regulating scientific research on an international basis.

The experiments involve the use of

newly discovered enzymes to transplant genes from virtually any living organism into viruses or bacteria, and the proposed US regulations, drafted last December but not yet finalised, would outlaw some of the more hazardous experiments and require that others be performed under special safety conditions. In particular, they specify that several types of experiments should only be carried out by transplanting genes into bacteria or viruses which have been genetically crippled to render them incapable of surviving outside an artificial laboratory environment.

Some members of the EMBO committee considered those regulations stricter than necessary to protect public health, but agreed that not all European countries should repeat the tortuous process of drafting their own regulations. So, with the proviso that the US controls are not made yet more stringent, the committee recommends that each country should establish its own national committee to vet applications for research grants involving gene transplant experiments and decide whether they conform to the US regulations. EMBO also suggests that an international committee, possibly under the auspices of the European

Science Foundation, should deal with problem cases.

One possible problem area involves the certification of crippled strains of bacteria or viruses. The US regulations detail the criteria by which modified microorganisms should be judged sufficiently crippled for use in gene transplant experiments, and one strain of bacterium recently produced at the University of Alabama is expected to meet the criteria. Although strains produced in the USA will be made available throughout the world, other modified strains may be produced in Europe.

National committees would be responsible for certifying whether or not such strains meet the criteria, but the EMBO committee is willing to offer its advice in specific instances. It has also offered to advise European governments and individual scientists about technical aspects of genetic manipulation research and safety precautions, a service that could be particularly useful for countries with small numbers of researchers.

While some European countries, including Britain, are already developing their own regulations and are unlikely to adopt the US version lock, stock and barrel, the idea of an international committee to discuss problem cases is likely to have considerable support throughout Europe. □

SOVIET JEWRY

Tailoring tactics to suit

Vera Rich reports from Brussels, where the Second World Conference of Jewish Communities on Soviet Jewry was held last week.

THE problem of Jewish scientists and other academics wishing to emigrate from the USSR to Israel continues to arouse debate. So too does the degree to which scientists should commit themselves to helping and campaigning for their disadvantaged colleagues. At the conference on World Jewry, a Special Commission on Science and the Humanities, chaired by Professor Yuval Neeman of the Israel Committee for Soviet Jewry, resolved to set up an International Federation of Concerned Scientists. Professor Denis Schiame of Oxford explained that its purpose would be to gather and disseminate information and to coordinate the activities of its affiliates from all countries where committees already exist or are being formed.

But the precise policy to be followed provoked lively discussion. A large

contingent, headed by the Nobel Prize-winner for Physics Dr Polykarp Kusch, stressed the importance of using international scientific cooperation agreements, which at present tend to favour the USSR, to exert pressure on behalf of disadvantaged scientists. The case of *refusnik* Aleksandr Lerner and the Fourth International Conference on Artificial Intelligence was cited: his attendance and active participation was secured after pressure was applied that threatened to change the venue from Tbilisi to a location outside the Soviet Union. But Dr Gabor Dessau of Pisa stressed the beneficial effect which foreign contacts could have on Soviet scientists who, he said, are often themselves secretly in favour of a greater freedom of movement. The idea of a total boycott of Soviet science was ultimately firmly rejected. It was agreed that the tactics to be used should rather be tailored to each specific problem as it arose.

Golda Meir referred to the "education tax" on Soviet academics, and cast doubt on the possibility of a mass

emigration of scientists seriously depleting the Soviet Union: dismissal was the immediate consequence of a visa application, she argued, so that "their brains are drained already". Aleksandr and Evgenii Levich, referring to the problem of classified information, recalled that their father, Academician Venyamin Levich, had been promised an exit visa at the end of 1975, when his knowledge would no longer be "classified"; this promise was later withdrawn.

Mention was also made of Soviet delegations to international conferences which regularly consisted of "representatives" chosen for their standing with the authorities and not their scientific knowledge or relevance.

The absorption of scientists from the Soviet Union into the Israeli economy, although not a direct concern of the conference, was discussed in various formal and informal gatherings. The current policy, said Dr. Eliezer Rafaeli, Rector of the University of Haifa, is to absorb them into existing institutions, establishing new departments or laboratories if necessary, but not setting up new universities. □

MEDITERRANEAN

Treaty in store

Sixteen Mediterranean states meeting in Barcelona earlier this month adopted a treaty on the protection of the Mediterranean against pollution which augurs well for a future "action plan". Robert Allen reports

AN interval of only two years separates discussion of the first principles of the Mediterranean anti-pollution treaty and its signature. There are two main secrets of this success, which comes despite the sharp political differences of the participants (Spain, France, Monaco, Italy, Malta, Yugoslavia, Greece, Turkey, Cyprus, Syria, Lebanon, Israel, Egypt, Libya, Tunisia and Morocco).

One reason is the existence of a regional scientific and resource management body, the FAO's General Fisheries Council for the Mediterranean (GFCM). Arabs and Israelis, Greeks and Turks, have worked together on the Council for some years now, and are used to each other at the scientific and technical levels of government. Secondly, there is the United Nations Environment Programme (UNEP), which can not only bring governments together, but can locate, harness and coordinate legal, scientific and planning expertise scattered amongst numerous research institutes throughout the Mediterranean area and within the UN's many (often competing) specialised agencies. It is this capability which is the key to an ambitious "action plan" being developed by UNEP and the Mediterranean coastal states and involving co-operative action on four related fronts: scientific, legislative, institutional, and planning.

UNEP has set up seven research projects*, to run initially for two years, in order to establish a basic pollution profile of the Mediterranean. Each project will employ a network of laboratories, coordinated by UNEP, appropriate specialised agencies and a designated national research centre. So far, eight countries (Algeria, Cyprus, Israel, Italy, Lebanon, Malta, Spain and Yugoslavia) have nominated a total of 19 laboratories. Other countries are expected to nominate laboratories soon.

Where necessary, UNEP will provide

*The 7 projects include: (1) baseline studies and monitoring of oil and petroleum hydrocarbons in marine waters (coordinated by UNEP and the Intergovernmental Oceanographic Commission, IOC, of UNESCO); (2) baseline studies and monitoring of metals, particularly mercury and cadmium, in marine organisms, and (3) of DDT and other chlorinated hydrocarbons in marine organisms—both coordinated by UNEP and the GFCM.

analytical equipment and training in its use. It will also fund a common maintenance service to avoid long gaps in coordinated data collection. The International Atomic Energy Agency's Marine Radiation Laboratory in Monaco will help ensure compatibility of results by setting monitoring standards and providing intercalibration services.

The entire scientific programme is expected to cost some \$10 million (almost \$2 million from UNEP, and about \$8 million from participating governments). For this, the coastal states of the Mediterranean will obtain a reliable pollution profile of the marine environment, derived from simultaneous measurements of pollutant levels in the same taxa, at a large number of sampling points, with the same instrumentation. There will be a comprehensive range of strictly comparable national data from the entire region instead of the present hodgepodge of hard facts, circumstantial evidence and anecdote. Without this data, planning the rational exploitation of the Mediterranean commons would be virtually impossible. It would also be extremely difficult to maintain the present rapid legislative progress when more contentious issues are tackled.

The treaty consists of a convention supported by two protocols (one on dumping, the other on cooperation in the event of pollution emergencies, such as oil spills), but the next one to come, controlling pollution from land-based sources like rivers and coastal outfalls, is the one most likely to founder if the data base is insecure. Mediterranean nations will be confronted with the substantial economic differences between north and south and difficult trade-offs between one sector of a national economy and another. With non-Mediterranean up-river states also involved, a reliable pollution profile will be essential.

Much of the additional promise of the action plan lies in its integrated planning effort, which aims to help governments develop the shared resources of the Mediterranean in a sustainable fashion. Permanent channels for cooperative planning are being set up, based on a conception of the Mediterranean Sea area as an ecological unit. It sounds slightly over ambitious, but as Peter Thatcher, Director of UNEP's Geneva office, remarked, the adoption of the treaty demonstrates that the "political leaders of the Mediterranean have overcome the divisive issues of today in order collectively to preserve their common heritage and discharge their responsibilities to future generations". □

CANADA

New breed

Canada's space programme was back on the nation's front page and television screens again briefly last month when the United States launched her neighbour's latest satellite, the CTS (communications technology satellite). David Spurgeon reports from Ottawa

CANADA'S space programme has been one of her most successful scientific and technological ventures. Alouette I, the first satellite designed and built by a nation other than the United States or the Soviet Union, was still sending back useful ionospheric data 10 years after its launch in 1962. Three other Canadian made scientific satellites successfully followed that (Alouette II, ISIS-I, ISIS-II), and two domestic communications satellites (Anik-I and Anik-II) both continue to serve the country's communications needs, the former being the first such satellite to be used in a geostationary orbit.

Canada's vast distances, severe climate and scattered population have all meant that its space program has been oriented toward electronic methods of communication. The new CTS satellite follows this pattern. It is considered the forerunner of a new breed of high-powered communications satellites that will be able to transmit directly to small, low-cost earth stations sometimes in remote and otherwise inaccessible locations.

The CTS is in fact the most powerful communications satellite now operating, by virtue of three major subsystems. One of these is the travelling wave tube amplifier: its power output of 200 watts operating at an efficiency of 50% compares with the six watts at 30% efficiency of the current generation of tubes used in communications satellites. A pair of lightweight extendable antenna arrays carries enough solar cells to provide one kilowatt of power to the satellite; and a three-axis stabilization system, employing a fixed momentum wheel and hydrazine gas thrusters, maintains the aiming accuracy of the antenna and keeps the spacecraft oriented toward the sun (rather than letting the spacecraft spin as is usual) to ensure full use of solar power.

The CTS satellite is able to employ high power transmitters because it operates in a new frequency band of 12-14 gigahertz allocated for broadcast satellites. Current systems operate in the 4-6 gigahertz band, which is shared by other types of system on the ground. For this reason, satellite power levels must be limited to prevent interference

USA

with ground systems: previously this was a major factor dictating the use of large and expensive ground antennas.

Antenna diameters for the CTS range from 32 inches to 30 feet. The only other satellite designed to transmit high-quality colour television to small, simple ground stations is the ATS-6 (applications technology satellite), which was launched in 1974 and is now being used by the government of India to relay educational programs to isolated villages. The ATS-6 must use a 30-foot antenna, and its effective radiated power is 53 DBW (decibel watts), whereas CTS's is 59 DBW.

Canadian groups have designed 26 experiments for the CTS, to begin in May. They include:

- One-way video and two-way audio transmission to provide continuing medical education for doctors in remote areas.
- High-quality telephone links to and from remote camp-sites in the James Bay area, where a power dam is being built.
- Curriculum-sharing between Carleton University, Ottawa, and Stanford University, California, using a digital video compression technique.
- Provision of computer-communications networks for native peoples in Canada's isolated northern regions, and evaluation of the potential.
- Linking of two francophone communities in different parts of Canada—Zenon Park, in Northern Saskatchewan, and a community in Quebec—for interchange of cultural and educational programs via two-way audio and video links. Other projects involve propagation measurements, a system for sharing telephone channels, and a fast frequency-shift keying system for high-speed digital data transmission.

The Canadian Broadcasting Corporation will try to demonstrate direct television reception from the satellite using simple, domestic-type equipment from laboratories in Japan and perhaps Europe. The satellite is also to be used for remote coverage of the 1976 Olympic Games equestrian competition at Bromont, Quebec. The signals would help in assessment of portable ground stations and reception and transmission from large, populated areas.

The United States and Canada will share equally in experiment time during the satellite's lifetime, using it on alternate days. If the experiments go as planned, the remote areas of many parts of the world—as well as Canada and the United States—may soon benefit from a multitude of communications services presently not economically feasible using conventional ground-based methods. □

With only one dissenting voice—that of Senator John V. Tunney of California—a subcommittee of the Joint Committee on Atomic Energy has produced a report giving full support to the liquid metal fast breeder reactor (LMFBR) programme and suggesting that “the time has come to end the discussion over whether or not [the United States] should have a breeder research and development program. Rather, national attention should be turned toward solving the outstanding problems associated with the program and its eventual commercialisation.”

Senator Tunney identifies what he describes as a failure to deal adequately with many fundamental issues and questions surrounding the breeder programme, and in particular he objects to the subcommittee's attempts to see discussion of the programme stifled. That would be an abdication of responsibility, he says, as well as inconsistent with the subcommittee's conclusion that the building of the Clinch River Breeder Reactor in Tennessee would not represent “an irreversible commitment to commercialisation.” (The Clinch River reactor, a 350-MW demonstration plant, is at present expected to be completed in 1983, at a cost of about \$2,000 million.)

The subcommittee, chaired by Congressman Mike McCormack of Washington, heard four main objections from opponents of the breeder reactor programme. It was argued that development costs would turn out to be prohibitive, that estimates by the Energy Research and Development Administration (ERDA) of indigenous uranium resources were too conservative, that energy demand would not grow as fast as projected, and that problems like plutonium toxicity and waste management would prove to be insoluble.

The subcommittee brushes those objections aside. One of its chief arguments in support of the LMFBR programme, for example, hinges on the suggestion that conventional reactors could start to run out of domestic supplies of uranium early in the twenty-first century, and unless the plutonium-producing capability of the breeder reactor is developed, the nuclear industry could grind to a halt. ERDA estimates, in fact, that there are about 3.6 million tons of uranium, available at a cost of less than \$30 per pound, in the United States. That is sufficient to guarantee a lifetime's fuel only to those reactors expected to be built before the mid-1990s.

- After 18 months of uncertainty, the future of Sacramento Peak Solar Observatory in New Mexico seems assured now that the National Science

Foundation (NSF) has agreed to provide \$1.45 million “to maintain the observatory at a productive level”. The US Air Force, which has operated the observatory since 1952, will, however, still maintain a presence, and provide some of that money—\$700,000 in the first year of the new arrangements, and \$450,000 in the second. After that it is not clear what, if any, contribution it will make, or how the NSF will make up the shortfall.

Even with the Air Force chipping in a further \$250,000 for the salaries of those remaining and \$250,000 in grants, the observatory will, according to its acting Director, Dr Richard Dunn, have to sustain cuts of 15% in its present budget of \$2.2 million and 27% in its staff of 63, as from June 30.

The new funding scheme was hammered out by a committee chaired by Professor Martin Schwarzschild of Princeton University, and the NSF has now formally accepted it. Agencies such as NASA and ERDA were also involved in the discussions: NASA already has a group working at Sacramento Peak, and the observatory's research programme contains sufficient plasma physics, hydrodynamics and so on for ERDA to find it relevant to its own fusion research activities. (In fact at one time it seemed as if ERDA might pick up the tab for the observatory itself.) As the Air Force's contribution diminishes, the NSF will undoubtedly be looking to other agencies to stump up some of the extra money needed. The matter awaiting resolution now is precisely how the observatory will be managed once the Air Force relinquishes its overall responsibility.

- The fifth annual report to Congress on marijuana and health shows that more than half of those between 18 and 25 have used the drug at some time, and that “what was once clearly statistically deviant behaviour has become the norm for this age group.” The report also says that the correlation with level of education has now all but disappeared. The authors of the report, produced by the Department of Health, Education and Welfare, say, however, that the drug's long term effects are not yet properly understood. Another aspect of the drug's use seems to have altered: whereas users of marijuana were once less likely than non-users to consume alcohol, they now seem more likely to do so. “Frequently the two drugs are used simultaneously,” says the report, “a combination that may be more hazardous than either used alone”.

Roger Woodham
Washington

IN BRIEF

Soviet treaty call

The Soviet Union called for an early treaty covering the development of weapons "still more pernicious" than nuclear arms when the 15th session of the Committee on Disarmament opened in Geneva last week. Delegates from 30 nations, urged to adopt a Soviet draft agreement on the issue, remained in the dark about the precise nature of the doomsday weaponry hinted at, but the Soviet delegate stressed that the reference was not to environmental or weather modification techniques, on which the USSR and USA are expected to approve a draft ban treaty by the end of the 1976 conference. The USSR also renewed its call for a committee of nuclear and non-nuclear states to discuss a comprehensive test ban treaty.

Nuclear safety

Three nuclear scientists who recently quit responsible positions with General Electric in a stand against the US nuclear programme last week told a

Joint Congressional Committee that many US nuclear installations should close because they are below Federal safety standards. In Europe, the Nuclear Energy Agency of the Organisation for Economic Cooperation and Development has meanwhile calculated that ambient radiation levels would increase by about 6% if electricity production needs were met entirely by fission reactors—an increase reckoned to pose less overall risk than hazards associated with existing methods of production. News has also come of a similar assessment attempted in the USA 20 years ago when, between 1945 and 1947, the US authorities secretly injected 18 terminally ill people with doses of plutonium equivalent to levels that would normally be received by plutonium workers in 100–800 years. Three of the human guinea pigs are still alive.

Nuclear arms

As a safeguard against possible terrorist attempts to seize nuclear fuel in Britain, a Bill presented to Parliament

last week extends the authorisation whereby security forces guarding UK Atomic Energy Authority establishments carry firearms to cover other nuclear installations housing "accessible" nuclear materials and UKAEA police accompanying fissionable material in transit. MPs unhappy about the Bill have already promised action at its second reading.

EEC fish limits

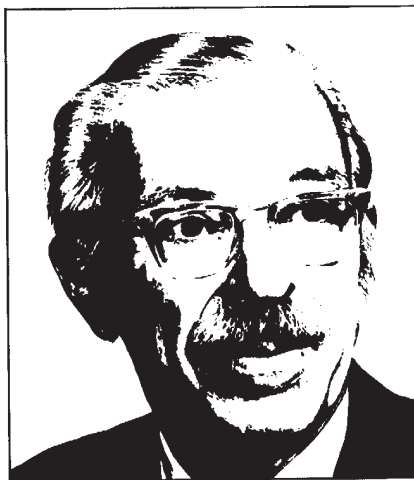
In a move anticipating an extension of international fishing limits during next month's Law of the Sea conference in New York the European Commission has called for the setting up of a 200 mile community limit around EEC states. Under the proposals, presented in Brussels last week, each nation will retain exclusive rights over a 12 mile strip within the wider community zone surrounding its own territory. A scientific and technical committee would advise fisheries officials of the Nine on necessary conservation measures before catch quotas and share-out proportions are drawn up for Community waters.

THE Delaney Clause of the Food Additives Amendment forbids the use of food additives that are carcinogenic, or animal feed additives that are carcinogenic and leave residues in the meat. The bureaucratic axe is being sharpened for bacon. On January 19 this year the FDA banned Red No. 2 (Amaranth), and on January 7 announced a forthcoming ban on diethylstilbestrol (DES) in beef production at an estimated cost of \$503 million annually to consumers. Representative James Delaney, evidently not satisfied with the effectiveness of his clause, introduced special and apparently superfluous legislation (HR 9837) for the latter purpose. Not to be outdone, the National Cancer Institute (NCI) recently warned pregnant women against eating beef liver.

Herbst and co-workers reported in 1975 on "benign alterations of the genital tract", especially abnormal vaginal mucosa, in a series of 110 females who had been exposed *in utero* when their mothers received DES prescribed during pregnancy. No cases of cancer were observed, although vaginal adenosis, which is suspected by some authors of being a "precancerous lesion", occurred in 35% of cases. The absence of cancer fits Herbst's earlier observation that the risk of cancer may be considerably less than 4 cases for 1,000 exposures. However, the dosage of DES received by the pregnant women is of extraordinary interest; it was

increased stepwise from the sixth to the 35th week for a total of 12.3 grams and a daily average of 60 mg per patient.

In other investigations, the "DES" content of liver from implanted cattle

Delaney's year**THOMAS H. JUKES**

was found to be 0.12 parts per 10⁶, so that 12.3 grams would be present in 100,000 tons of contaminated liver. The total amount of beef liver produced in the USA is about 140,000 tons per year. More than a century of beef production would have been needed to supply DES in beef liver to the women in the study, none of whom produced offspring who deve-

loped cancer. This assumes that the "residues" are actually DES, which is now known not to be the case. No wonder Mr Delaney is impatient for his ban; he may be worried that common sense will take over.

In a speech in the Senate, calling for a ban on DES in beef production, Senator Edward Kennedy said the ban had been requested by Dr Frank Rauscher, Director of NCI, and that public funds of about \$700 million annually were furnished to NCI, which provides scientific guidance to him on questions of cancer. Perhaps a small allocation, say \$100, should be set aside for the purchase of a pocket-size calculator to compute the actual risk from a daily intake of one molecule of DES, which NCI scientists have testified might cause cancer. Part of the \$100 could be used for a textbook on nutrition, so that NCI could learn that beef liver is the best dietary source of folic acid, and that folic acid deficiency is common during pregnancy.

Indeed, folic acid was discovered, as a result of the anaemia in pregnant women caused by its deficiency, by Dr Lucy Wills in 1931. The deficiency is still prevalent throughout the world, as noted by the World Food Congress in Rome, 1973. A continuation of the dietary lack of folic acid in pregnancy will be aided by NCI's recommendation against consuming beef liver. The protective effect against cancer of this recommendation seems dubious.

news and views

Receptor maps for viruses

from Arie J. Zuckerman

VIRUSES are not motile and they rely on such processes as diffusion and transport within body fluids to bring them into contact with susceptible cells. The critical step in successful viral infection is the susceptibility of cells, and the means by which viruses are incorporated into host cells has been the subject of much research. The susceptibility of cells is usually determined by early steps in virus-cell interactions which include the attachment and penetration of the virions and the release of the viral nucleic acid in the cells. With some animal viruses such as the influenza viruses and some of the picornaviruses such as Cocksackie virus and poliovirus, attachment is associated with the presence of specific receptors for viral adsorption on the cell surface, but whether receptors are required for all viruses is not known. The presence or absence of receptors on the cell surface depends on several factors which include the particular type of tissue or organ, stage of maturity, changes in the physiological environment such as culture *in vitro*, and genetic factors involving cellular rather than immunological mechanisms.

On the other hand, there are also viruses, including arboviruses, poxviruses and herpes viruses, which will grow in a variety of cell types, and therefore there appears to be little specificity about the adsorption process of such viruses. In these instances a mechanism such as phagocytosis or viropexis may be important in the incorporation of virus into susceptible cells. Another mode of viral entry entails fusion of the viral envelope with the plasma cell membrane. Fusion takes place by a process of alignment of the two membrane structures, followed by the formation of a channel between virus and the cytoplasm through which the nucleocapsid passes into the cell. A more recently described mode of entry of virus into the cell is by a process of membrane fission rather than by viropexis.

Lonberg-Holm and his colleagues point out on page 679 of this issue of

Nature that the specific receptors for several nonenveloped viruses are present on cells in limited number and therefore these can be saturated with excess virus. They report results of experiments in which the attachment to HeLa cells of highly purified virus particles radiolabelled with carbon, sulphur and phosphorus was measured. Four receptor families were found. The first family contains receptors to human rhinovirus types 2, 1A and 1B. The second family contains receptors to human rhinovirus type 14 and Cocksackie A21 and a number of other rhinovirus serotypes. The third family contains poliovirus, and the fourth contains Cocksackie B3 and probably the other Cocksackie B serotypes as well as several serotypes of adenovirus. It is reasonable to consider that receptor specificity influences virus tropism in the tissues thereby contributing to the patterns of pathogenesis of virus infections. An example is the finding that Cocksackie A21 shares receptors with a large number of human rhinoviruses and this may be related to the ability of Cocksackie A21 to cause coryza. Another example is that both Cocksackie B viruses and adenoviruses produce persistent infections in HeLa cells cultured in the presence of human serum.

Burrows pointed out, however, (*Microbial Pathogenicity in Man and Animals*, 303-332, Cambridge University Press, 1972) that many investigators have attempted to associate characteristics of virus growth and behaviour *in vitro* with virus behaviour in the intact host. Characteristics such as tissue receptor activity, the production of or susceptibility to interferon, growth and cytopathogenicity in cultured cells and virulence for laboratory animals have all been linked to differences in virulence for the normal host. The determinants of virulence, although clearly related to those of virus growth, differ markedly between viruses and indeed between strains of the same virus. It was Chaproniere and Andrewes (*Virology*, 4, 351; 1957) who

stressed that the ability of many viruses to grow in monolayer cell cultures derived from tissues or hosts which are not susceptible to the virus, limits the usefulness of such cultures for basic studies of susceptibility as they relate to the intact host.

It is generally considered that the morphological and functional capacity of cells maintained in organ culture may offer a system for cultivating viruses which closely simulates conditions in the intact host. Examples of the specificity of the effect on the target organ and differential susceptibility of different hosts are found within each class of viruses and organ cultures have reproduced many, but not all, of the phenomena of specificity observed in the intact animal. Extension of the studies reported by Lonberg-Holm and his colleagues and the precise identification of the properties of the specific virus receptors may provide a key to the understanding of viral pathogenesis and perhaps also to the prophylaxis and even therapy of virus infections. □



A hundred years ago

After discussing the results obtained by Stoney, Thompson, and Clerk-Maxwell, mainly derived from the properties of gases, I come to the conclusion that in the present state of our knowledge the best approximation that we can make to the size of the ultimate atoms of matter is the mean of their determinations. I adopt for simplicity $\frac{1}{1000}$ th of an inch as the unit of length, and $\frac{1}{1000}$ th of an inch cube, or $\frac{1}{1000000000}$ th of a cubic inch, as the unit of volume. In the case of a true gas the number of atoms in the length of $\frac{1}{1000}$ th of an inch at 0 °C, and a pressure of one atmosphere, would be 21,770, and hence, in $\frac{1}{1000}$ th of an inch cube, about 10,320,000,000,000.

From *Nature*, 13 (February 24), 332; 1876.

Charm: what else could it be?

from W. T. Toner

THE peculiar bubble chamber event cautiously put forward by the European "Gargamelle" collaboration as possible evidence for charm now has two companions (Deden *et al.*, *Phys. Lett.*, **58B**, 361; 1975; Bleitschau *et al.*, *Phys. Lett.*, **60B**, 207; 1976), and four similar events have been found in pictures from the fifteen foot diameter bubble chamber in the neutrino beam at the Fermi National Accelerator Laboratory (FNAL) near Chicago. One event could always have been a freak chance but not three, still less seven. A new physical effect has been dis-

covered.

All seven events are of the type shown in Fig. 1, where an unseen neutrino from the beam interacts with a nucleus in the chamber liquid to produce several particles one of which is, as usual, a negative mu-meson. Two of the other particles are uncommon: a positron and a neutral V particle, so called because of the pattern made by the tracks from its decay a few centimetres from the main interaction in which it was produced. The Vs are members of the group of "strange" particles. The positron signals the

weak (radioactive β^+) decay of some particles whose lifetime must be extremely long on the subnuclear time-scale as otherwise the decay would be strong. But since the positron appears to come directly from the interaction point the decay must take place within a millimetre and the lifetime must therefore be significantly less than that of the Vs. No known particle has a lifetime in this range although some of the hypothetical charmed particles should have, and should frequently involve strange particles in their production and decay.

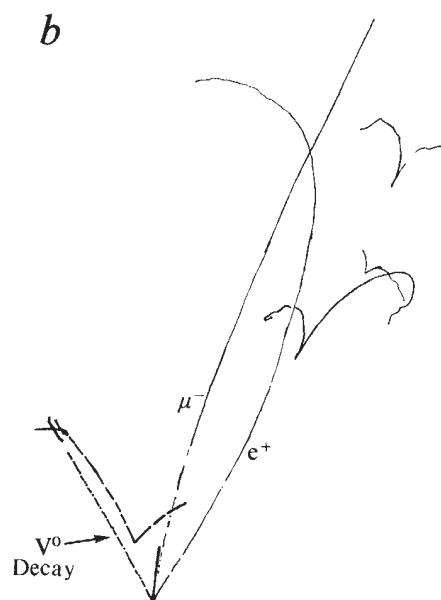
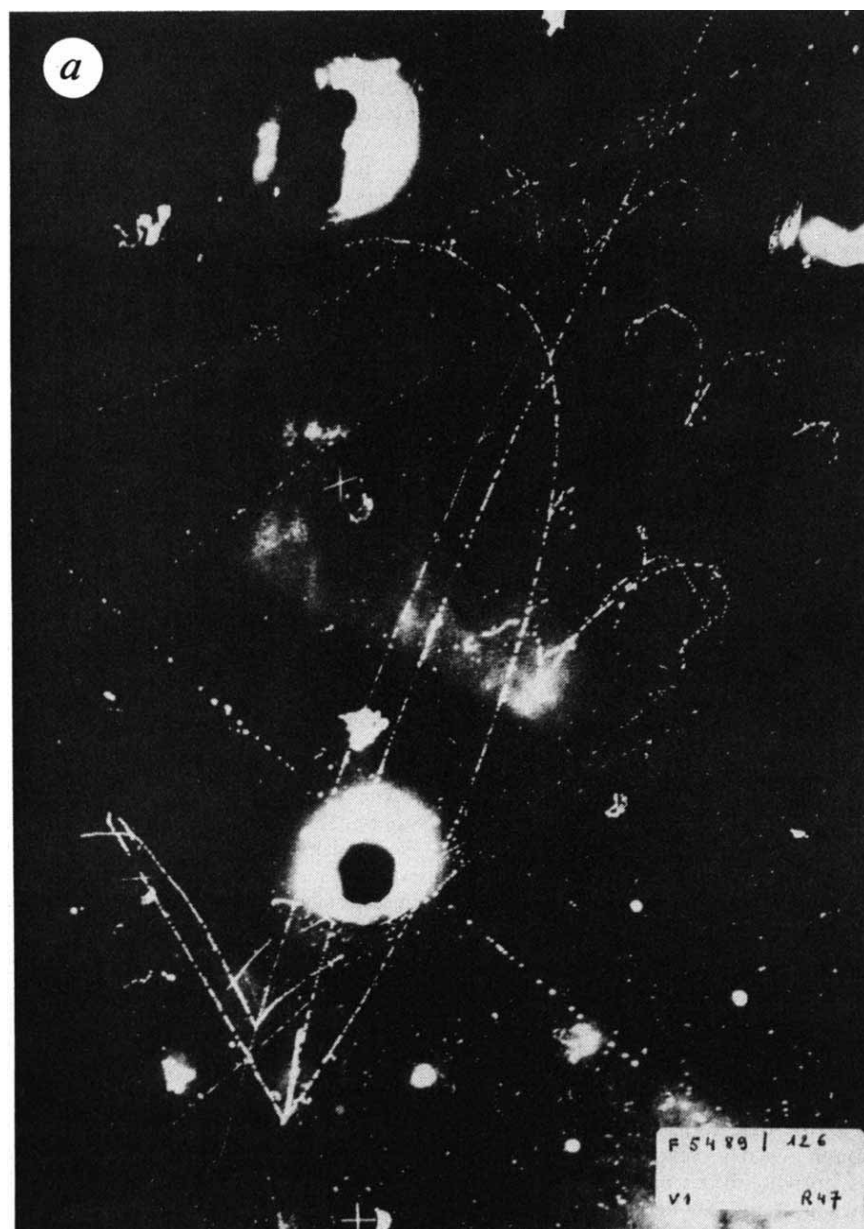


Fig. 1 *a*, One of a stereoscopic set of photographs of an event in the Gargamelle bubble chamber at CERN. Only charged particles leave tracks. The neutrino beam enters from the bottom of the picture. *b*, Sketch of the tracks involved in the event. (Stereoscopic reconstruction eliminates the others.) The mu-meson (μ^-) leaves the chamber without interacting; the positron (e^+) has a rapidly changing curvature and is associated with electron-positron pairs; the V^0 itself leaves no track, what is seen are the two tracks from its decay. The other two particles emitted in the primary interaction are unimportant to the interpretation.

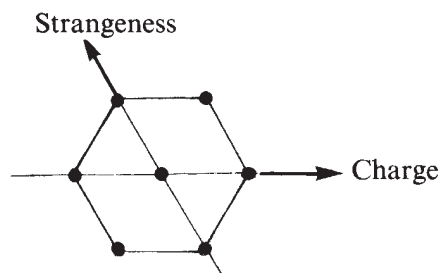


Fig. 2 A two-dimensional pattern in charge-strangeness space. There are three particles at the origin and one at each of the six corners.

What is charm or for that matter, strangeness? Both are properties akin to electric charge which are supposed to be possessed by elementary particles in quantities of $0, \pm 1 \dots$ units. Everyday particles like the proton and neutron have charm and strangeness zero. Like electric charge, the total amounts of charm and strangeness must be conserved in all strong (nuclear) or electromagnetic interactions but unlike electric charge, neither need be conserved in β -decay or other weak processes.

Strangeness has been an accepted fact for more than 20 years. The concept was invented in 1952 by Pais (*Phys. Rev.* **86**, 663; 1952) to explain why the V-particles discovered by Rochester and Butler (*Nature*, **160**, 855; 1947) should be copiously produced and yet decay only slowly. Copious, strong, production of pairs having opposite and mutually cancelling strangeness would be allowed; but all particles die alone, and the metamorphosis from strange back into ordinary matter could take place only slowly, by weak decay.

The idea was enormously successful and is essential to the SU_3 or Quark classification scheme which fits all particles known up to a year ago into triangular and hexagonal patterns such as that in Fig. 2 and which interrelates their properties in like manner. Electric charge is one axis of the pattern and strangeness the other. Charmed particle production and decay would obey similar rules and charm would add a third dimension to the patterns, as in Fig. 3. The most obvious experimental consequence would be the existence of the many new particles in the third dimension, with charm $\pm 1, \pm 2$, whose decays into uncharmed matter would be weak, and of a few new particles composed of pairs of oppositely charmed quarks with net charm zero. These might or might not have long lifetimes.

In the case of charm, the theory came before the experimental evidence. It was invented by Bjorken and Glashow in the spirit of exploring the consequences of adding a new dimension to the patterns (*Phys. Lett.*, **11**, 255; 1964). Charm remained a theoretical toy which predicted too many new effects until the Gargamelle collaboration found another new class of neutrino interactions in which the neutrino did not turn into a charged mu meson as usual, but retained its identity (Hasert *et al.*, *Phys. Lett.*, **46B**, 138; 1973). It was difficult to reconcile this observation with the known absence of equivalent processes among strange particles without invoking charm and the elegantly constructed cancellation mechanism of Glashow, Iliopoulos and Maiani (*Phys. Rev.* **D2**, 1285; 1970) which linked charm with strangeness. A consequence was that this link should show up by the frequent appearance of strange particles in charm non-conserving weak decays and in the weak production of single charmed particles by neutrinos.

In the summer of 1974, charm was an exciting theoretical conjecture. Enthusiasts, such as Iliopoulos, were willing to wager a case of wine on its imminent discovery (*Proc. XVII Int. Conf. on High Energy Physics*, (edit. by Smith, J. R.) III-100 SRC, 1974), though others remained sceptical. A few months later the ψ (or J) particles were discovered and found to have many of the properties forecast for the charm $=0$ or "hidden charm" members of the family. Although alternative explanations for the ψ s were and are still tenable it seemed to most that definite proof of the existence of charm was very near. That seems even more surely the case now, but the last stages of the search have proved difficult.

The only direct evidence other than the ψ s has come from experiments with neutrinos, which interact so rarely that their mean free path is hundreds of Earth diameters. Any apparatus designed to observe large numbers of interactions is necessarily massive, and is clumsy in even the most skilled hands. A group using a detector weighing several hundred tons composed of iron slabs interleaved with scintillation counters and spark chambers reported two "di-muon" events at the 1974 conference (see C. Rubbia, *loc. cit.* IV-119) and were later able to show with many more events that weak decays were taking place among the products of neutrino interactions at a rate which could not be explained by any known particle (Benvenuti *et al.*, *Phys. Rev. Lett.* **35**, 1199; 1975). But although many events had been seen and the effect was by then undisputed,

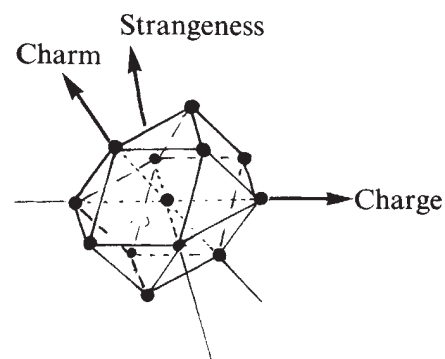


Fig. 3 A three-dimensional pattern in charge-strangeness-charm space. There are four particles in the centre, at the origin, and one at each of the twelve corners.

charm was a very plausible rather than a necessary interpretation.

The new bubble chamber events clearly establish the expected link with strangeness and set loose bounds on the lifetime of the new particles but even their graphic detail is insufficient to determine the precise nature of the interaction because in each event there are two neutrinos whose energies are unknown: the one which initiated the event and a second one emitted in an unknown direction from the β -decay. Without mass values and decay schemes one cannot begin to construct the pattern to see if it fits with charm, let alone make the sophisticated tests needed to prove that it solves the theorists' problems.

The next step is to find cases where the decay does not involve a second neutrino in order to establish the masses of the supposed charmed particles. Events of this type will be harder to identify because they will also lack the positron which singles out the weak decay. One such event with other strikingly characteristic features of charm has been reported (Cazzoli *et al.*, *Phys. Rev. Lett.* **34**, 1125; 1975) but subsequent experiments with greater sensitivity have failed to reveal others of the same kind. It may be necessary to collect many events with less obvious distinguishing features and search in the mass distributions of different combinations of supposed decay products for narrow peaks, which would be alternative evidence for the existence of particles with unusually long lifetimes. This is a sure and well-timed method of finding new subnuclear states although it takes time and patience. Meanwhile, the commonest answer to the question what else it could be is: something else very like charm. $\square$

Bacterial cytochromes *c* structure

from D. R. Thatcher

COMPARISONS of protein structure at the three-dimensional level are able to distinguish similarities, and therefore probable evolutionary connections, which would not be convincing at the amino acid sequence level. The best example of the application of this approach is the discovery of the characteristic super-secondary structure of enzymes which bind dinucleotides. Now that the X-ray crystal structure of four different types of cytochromes *c* is known, a detailed three-dimensional comparison has been made (Dickerson, Timovich and Almasy, *J. molec. Biol.*, **100**, 473; 1976), and the evolutionary implications discussed.

Eukaryotic cytochromes *c* form a clearly homologous group of proteins, both at the three-dimensional level (horse, bonito and tuna crystal structures are available and show an almost identical folding pattern) and at the amino acid sequence level (sequences from over 70 different eukaryotes have similar lengths and have a minimum of 25% of their sequence identical). By contrast bacterial cytochromes *c* show a formidable variation in chemical structure and physical properties; a reflection of the extreme antiquity and diverse modes of energy metabolism in this group of organisms. Dickerson *et al.* have compared the already published three-dimensional structures of tuna cytochrome *c* and *Rhodospirillum rubrum* cytochrome *c*₂ (a purple non-sulphur bacterium) with the new structures of cytochrome *c*₅₅₀ from *Paracoccus denitrificans* and *Pseudomonas aeruginosa* cytochrome *c*₅₅₁.

All four cytochromes differ significantly in the length of their polypeptide chains (cytochrome *c*=103 residues; *c*₂=112 residues; *c*₅₅₀=134 residues and *c*₅₅₁=82 residues).

Although the variation in amino acid sequence between these cytochromes is large (in the case of *c*₅₅₁ primary structural similarity can barely be discerned), the overall folding pattern around the haem prosthetic group is recognisable in all four cytochromes. The bacterial cytochromes mainly differ from the tuna cytochrome *c* by additional residues in the external loops around positions 53 and 75 (cytochrome *c* numbering) whilst *c*₅₅₀ also has an insertion in a loop in the 20's region and also possesses a 15-residue tail. The small size of *c*₅₅₁ is due to a massive deletion (39–58 in tuna cytochrome *c* numbering) of a loop at the bottom of the molecule. The folding of

the rest of the molecule is however conserved and *c*₅₅₁ is clearly related to the other cytochromes.

Dickerson *et al.*, go on to propose that this same deletion probably occurs in cytochrome *f* (involved in algal photosynthesis), *c*₅₅₅ (a photosynthetic cytochrome of the green sulphur and purple sulphur bacteria), *c*₅₅₃ (from the sulphate respirer *Desulphovibrio*) and the *c*₅ of *Pseudomonas mendocina*. This assumption makes the sequence alignment of all these cytochromes with eukaryotic cytochrome *c* much more credible and suggests the existence of a large evolutionarily homologous group of cytochromes whose members all possess a similar polypeptide folding pattern (the cytochrome fold). It is tempting to assume that the different electron transport pathways in which these diverse cytochromes operate also have an evolutionary connection and Dickerson *et al.* explore the way in which their data can be interpreted in terms of the development of bacterial energy metabolism.

Dickerson *et al.*, have drawn attention to the remarkable sequence work of Ambler and colleagues (Ambler *Handbook of Biophysics and Molecular Biology*, 1976; Ambler, Meyer and Kamen, *Proc. natn. Acad. Sci. U.S.A.*, in the press) on other cytochromes *c*₂ in the purple-non sulphur bacteria. Some of these *c*₂'s align almost exactly with eukaryotic cytochrome *c* (*Rhodomicrobium vannelli*, for example) whilst other *c*₂'s have, like *Paracoccus* *c*₅₅₀, additional residues around residues 20, 50 and 70 (such as *Rhodopseudomonas capsulata*). In fact this range of structural variation within *c*₂ has now been extended by the finding of Ambler and Meyer (personal communication) that the *c*₂ of *Rhodospirillum tenue* has the massive deletion characteristic of *c*₅₅₁. We are then left with the intriguing observation that the range of structural variation within cytochromes *c*₂ of the purple non-sulphur bacteria is as great as in all other cytochromes possessing the cytochrome fold.

Dickerson *et al.* consider that the main evolutionary implication of this homology is that bacterial and eukaryotic respiration arose from the dual function cyclic photophosphorylation and respiratory electron transport chain of purple non-sulphur bacteria by loss of the photosynthetic capability.

This interpretation confirms previous schemata for prokaryote evolution (such as Hall, *J. theor. Biol.*, **30**, 429–454; 1971 and in the *Evolution of Bioenergetic Processes*, edit. by Broda, E., 1975) but the evidence really hinges on the question of whether the different cytochromes *c* are evolutionarily representative of the whole electron transport pathways of which they are

members or whether this phylogeny has been muddled by genetic transfer. Physiological studies within the purple non-sulphur bacteria, which have representatives of each major type of cytochrome should resolve this question to some extent. Nevertheless, the purple non-sulphur bacteria could be the key to our understanding the molecular evolution of cytochrome *c*.

Palaeomagnetic diversity

from Peter J. Smith

PALAEOMAGNETIC studies were begun early this century in an attempt to determine the characteristics of the Earth's magnetic field before the few hundred years of direct observation. By the 1960s, however, they had come to be associated more particularly with the growing evidence in favour of continental drift and seafloor spreading. With the now-widespread acceptance of the principal tenets of the new global tectonics, there has been a tendency for palaeomagnetism to revert more frequently to its original aims, although it is still used widely to elucidate possible continental and/or polar movements (especially those preceding the onset of Wegenerian drift) and has, in addition, become a useful tool for solving particular geological problems (for example, in stratigraphy).

This modern diversity of roles is well illustrated by the many reports that have appeared in the past few months alone. Roy *et al.* (*Geophys. Res. Lett.*, **2**, 537; 1975), for example, have carried out a straight palaeomagnetic directional study of the world's oldest red beds—argillites, 2.3 billion yr old, found in the Huron Supergroup of Ontario. Perhaps 'straight' is hardly the right word in this context, for it is seldom easy to obtain a reliable direction from rocks of such an age. In this case, however, a combination of thermal, chemical and alternating field cleaning appears to give a reliable ancient pole at 67°N, 158°E from a magnetisation acquired at, or within a few tens of millions of years of, the formation of the beds. The new pole is ~100 Myr older than the oldest previous Laurentian poles; and its significance is that in the context of previous poles it provides evidence that the apparent polar wandering path relative to Laurentia from 2.3 to 1.9 billion yr ago was mainly latitudinal and from north to south.

Paradoxically, not only can it be

difficult to obtain a good direction from very old sediments because of their age, it can be equally difficult to obtain a good direction from very young sediments because of their extreme youth. The problem is that the most recent sediments are still unconsolidated and thus subject to erosion, slumping and many other processes likely to distort whatever magnetisation they may already have acquired. Moreover, the 175 1-m cores taken from the top of the sediment in Lake Geneva by Creer *et al.* (*Earth planet. Sci. Lett.*, **28**, 127; 1975) were also disturbed during handling and kept in storage for more than a year before any palaeomagnetic measurements were made on them. It is thus all the more surprising that no less than 39 of these cores gave "satisfactory and interpretable" declination-depth curves. Comparison of these results with the historic-archaeomagnetic declination curve has enabled Creer and his colleagues to estimate the sedimentation rate and its variation in Lake Geneva over the past 400 yr.

By contrast, Thompson's (*Geophys. J.*, **43**, 847; 1975) measurements of magnetic declination in lake sediments have been concerned with a much longer time scale. Cores from Lake

Windermere, Blelham Tarn and Ennerdale Lake in NW England and from Lough Neagh in Northern Ireland show that an oscillation in declination (about true north) with a period of about 2,700 yr has been occurring for at least the past 10,000 yr. Such oscillations are probably due to a combination of dipole wobble, drifting non-dipole sources and stationary intensity-varying non-dipole centres—which presumably explains why declination-time curves from Britain, Switzerland, the Aegean Sea and the Black Sea are difficult to correlate. However, as long as the core magnetisation is stable and at least one horizon is dated absolutely or cross-correlated, recent NW European sediments may be dated against the British declination mastercurve.

Thompson's sediments were all normally magnetised, of course; it is necessary to go back a few more thousand years to the most recent (short) reversal. Not that the reversal pattern is yet perfectly clear, even for the past few million years. For example, the Olduvai normal event within the Matuyama reversed epoch was originally dated at ~ 1.9 Myr and the subsequently discovered Gilsa event in the same epoch was dated at about 1.6 Myr. In short, there appeared to be two separate events—a view sup-

ported by an early report suggesting that the Gilsa type section in Iceland included two normal flows possibly separated by a reversed one.

Unfortunately, it has since become clear that the original Olduvai date may not be accurate; indeed, the wide limits now placed on it are such as to throw doubt on the whole existence of two separate events. This doubt has now been reinforced by Watkins *et al.* (*Earth planet. Sci. Lett.*, **27**, 436; 1975) who have re-examined the Gilsa section and find that although there are two normal flows there is no reversed flow between them. Thus only one normal event is definitely represented (at 1.58 ± 0.08 Myr); and the question of whether the Gilsa and Olduvai events are identical or distinct remains open.

Returning now to the almost single (normal) polarity of the Brunhes epoch, Watkins and Richardson (*Geophys. J.*, **43**, 501; 1975) have been looking again at the contrast in palaeomagnetic data between the northern and southern hemispheres. Second-order differences between hemispheres were first discovered by Wilson (*Geophys. J.*, **19**, 417; 1970 and **22**, 491; 1971) who concluded that during the Upper Cretaceous the axial dipole was displaced along the rotational axis

ONE cannot help admiring the way certain species of plant and animal have exploited to their own advantage situations created by man. Even the most ugly and destructive activities have provided opportunities for those species with a sufficiently wide range of tolerance or the capacity to evolve such a tolerance. As a result urban environments have become populated by species such as the starling whose cliff and tree nesting and roosting habits, coupled with the capacity to feed in short turf, have provided it with a competitive opportunity. Among plants, the rose bay willow herb (*Chamaenerion angustifolium*) is a particularly successful invader of demolition sites, arriving as wind borne seeds and propagating vegetatively once established by means of stolons which give rise to erect stems. It is intolerant of shade and its natural habitat is open scree slopes. This is one of the many weed species which has survived in open localities since the close of the last glaciation and has spread extensively as man has provided it with new opportunities.

The mining of heavy metals and the tipping of spoil has created habitats which are toxic and inhospitable to most plant species, but some appear capable of evolving tolerance to such conditions and expand their populations as a result. For example, the bladder

campion (*Silene vulgaris*), another periglacial species turned weed, has developed races tolerant of high zinc concentrations and able to invade mine spoil (Gries, *Flora, Jena*, **156**, 271; 1966). A similar process has been observed operating in a number of species, particularly grasses, in the British Isles (Bradshaw *et al.* in *Ecology and the Industrial Society*, edit. by Goodman, G. T., Edwards, R. W., and Lambert, J. M., 327, Blackwell, Oxford; 1965).

Orchids are not normally regarded as a group of plants exhibiting heavy

Orchids adapting

from Peter D. Moore

metal tolerance, but some recent observations by Richards and Swan (*Watsonia*, **11**, 1; 1976) in Northumberland in the north of England have altered this. They have found the narrow-lipped helleborine (*Epipactis leptochila*) growing on eleven sites, 180 miles north of the nearest previously recorded station. All the sites consisted of bare, open river gravels and most of them contained very high levels of zinc (up to 2,300 p.p.m. air-dry soil), and had a neutral pH.

The characteristic habitat of *Epi-*

pactis leptochila is beneath beech canopies on chalk and limestone in southern Britain. The plants in Northumberland could be derived from some previously undiscovered, relict population, or they could have invaded directly from the south of England. Richards and Swan consider it possible that the species has been in the area for 80 yr or more, having re-examined older, less critical identifications, but the origin of the species in the area remains uncertain. What is of greater importance and interest is the development of tolerance to zinc and its resultant success in polluted gravels where mining activities have occurred. At such sites the general vegetation is sparse due to toxicity, hence there is little competition from other herbaceous species, which is one character these northern sites have in common with the southern habitat of *Epipactis leptochila*.

As Bradshaw *et al.* have shown, not all plant species possess the ability to adapt to soils polluted with toxic metals, but evidently *Epipactis leptochila* does, and the Northumberland populations must have been subjected to considerable selective pressure for zinc tolerance. Like the starling and the rose bay willow herb, this is a species with latent talents that is now proving its worth by exploiting habitats made derelict and inhospitable by man. □

to the north by 285 ± 74 km. Later, however, Watkins (*Geophys. J.*, **28**, 193; 1972) pointed out that, although the northern hemisphere palaeomagnetic results for the Brunhes alone are consistent with a dipole offset to the north by 300–400 km, the southern hemisphere data require no such offset. He therefore suggested that the simplest model consistent with data from both hemispheres would have a main centred dipole with a weaker axial dipole some distance to the north.

In a more thorough analysis, Watkins and Richardson now show that the Brunhes palaeomagnetic data are best satisfied by a major dipole offset to the north together with two minor axial dipoles offset arbitrarily to the core-mantle interface (one in each hemisphere). These minor dipoles have moments of 1–4% of that of the main dipole, the one in the southern hemisphere having the same polarity as the main dipole and the one in the northern hemisphere opposing the main dipole. This is apparently the simplest model consistent with all current Brunhes palaeomagnetic results although more complex models could be devised (and may be necessary later to explain better data).

Finally, Bhattacharyya and Leu (*J. Geophys. Res.*, **80**, 4461; 1975) have used the magnetism of rocks indirectly to investigate a much more local effect. From an analysis of magnetic anomalies over Yellowstone National Park, they show that the Curie point isotherm below this geothermal area is particularly shallow. This is only to be expected and in any case has been proved before using other methods. The point of this confirmation, however, is its suggestion that aeromagnetic data may prove useful in regional reconnaissance for potential geothermal energy resources. □

Tail-wagging antibodies?

from C. C. F. Blake

Now that X-ray studies have established a broad understanding of the antigen binding function of immunoglobulins, attention is being turned to the Fc region and how its complement fixation and B-cell activation functions are influenced by binding of antigen.

The first successful structural study of a whole myeloma protein, the IgG1 Dob, by both low resolution X-ray analysis and electron microscopy (Sarma *et al.*, *J. biol. Chem.*, **246**, 3753; 1971; Lebow and Davies, *J. ultrastr. Res.*, **40**, 349; 1972) revealed that the molecule was T-shaped, but little else. Recently Huber and his

colleagues (Colman *et al.*, *J. molec. Biol.*, **100**, 257; 1976) reported a second low resolution study, of the IgG1 Kol, which has produced much more information. There are two reasons for this: first, high-resolution structural information on the Fab region is now available and second, the Kol protein does not have the deletion in the Fab–Fc hinge region that the Dob protein has. Since the resolution of Huber's map is only 5 Å, only the quaternary structure of the molecule—the arrangement of the various domains—can be analysed in any detail. Changes at this level of structure, however, could be highly significant, and some very intriguing results have been obtained, even though, or rather because, there is no electron density for the Fc region of the molecule.

One of the characteristics of X-ray analysis of crystal structures is that it is only possible to see side-chains or parts of molecules that are positionally stable in the crystal. Thus the lack of electron density in that part of the crystal that should contain the Fc of Kol, must indicate that the whole region can, and does, take up at least two orientations relative to the well-defined Fab regions. Huber has interpreted this as indicating the presence of a hinge in the molecule at the point at which the electron density fades out—immediately C-terminal of the inter-heavy chain disulphide bridges. If this is so the hinge is different from the classical one proposed to account for the Y→T transformation which must be N-terminal of these bridges. It should be noted that the angle between the two Fab arms is 125° in Kol, in contrast to the 180° found in Dob.

Yet a third hinge region may be present in the molecule, coincident with the switch regions between the V and C domains of the Fab arms. Interpretation of the Fab region of the Kol protein in terms of the known structures of the human V_k dimer fragment Rei, and the mouse McPC 603 Fab fragment, has shown that the V and C domains are not in the same relative orientation as that found in the Fab fragments so far analysed. The difference can be judged by the angle of intersection of the pseudodyads that relate the light and heavy chains in the two domains, which changes from 120° in the Fab fragments to 170° in the Kol protein. Colman *et al.* refer to this change as “bending the elbow” of the Fab arms—in the Kol protein therefore the arms are almost straight.

Huber and his colleagues speculate that these two new hinge regions may permit antibodies to act as allosteric proteins, in which the allosteric effect is transmitted through the polypeptide chain from one domain to another.

They suggest the possibility that hapten binding is accompanied by a “bending of the elbow” and that the elbow movement is sensed by the hinge causing a change in the Fc region that by implication influences complement fixation. Although there is little other evidence that complement fixation is accompanied by conformational changes, the present hypothesis is certainly plausible in view of the extraordinary domain structure of the immunoglobulins, and we now await the results of hapten binding to the Kol protein with considerable interest. □

Tunguska revisited

from David W. Hughes

At about 7.17 a.m. local time on June 30, 1908 in the basin of the River Podkamennaya Tunguska, Central Siberia (latitude 60° 55'N, longitude 101° 57'E) a gigantic explosion occurred. The ancient trees of the mighty Yenissi taiga were torn up by their roots and in places piled up in thick layers by the explosion wave, their trunks pointing radially away from the centre of the explosion. The devastation extended over an area of radius 30–40 km, the centre of the area having been ravaged by fire, searing being traceable for 18 km. A farmer 60 km away told how his shirt was almost burnt off his back, the explosion throwing him off the steps of his house and several feet across the ground. Eye witnesses up to 500 km away saw, in a cloudless sky, the flight and explosion of a blindingly bright, pale blue bolide, “which made even the light of the Sun appear dark”. This left in its wake a thick dust trail. The explosion took the form of a vertical column of fire and threw incandescent matter up to a height of 20 km. The sound of the explosion, like gun fire, reverberated thousands of kilometres away, seismographs registered an earthquake; the explosion air wave, recorded on microbarographs in many meteorological stations, went twice round the world. Magnetic disturbances similar to those subsequently recorded after atmospheric nuclear explosions were recorded at the Irkutsk Observatory.

After the explosion the nights were exceptionally bright over Western Asia and Europe, the enhanced night brightness slowly diminishing and disappearing after two months. In mid-July, two weeks after the explosion, the coefficient of transparency of the atmosphere was found to be noticeably depressed over California. This, it was suggested, was due to the loss of vast amounts of material from the incident body as it

travelled through the atmosphere. Observations indicated this loss to be several million tons, a hundred times more than the normal annual influx of meteoric matter.

Fortunately the fall region was very sparsely populated, the only inhabitants being scattered bands of nomadic Evenki reindeer herders. Due to the untimely occurrence of political upheavals in Russia the first cursory inspection of the impact site did not take place for 19 years when eventually Leonid A. Kulik headed an expedition organised by the Academy of Sciences of the USSR.

Many expeditions have since visited the area and many papers have been written discussing the phenomenon and putting forward ideas as to its cause. One of the latest papers appeared in a recent issue of *Physics of the Earth and Planetary Interiors* (11, 61; 1975). In it Ari Ben-Menahem of the Adolpho Bloch Geophysical Observatory, Rehovot, Israel re-analyses the old seismograms of the Tunguska event and deduces the parameters of the original explosion by comparing the old data with contemporary records of the seismic and acoustic effects of the air explosion that took place at Lop-Nor, Sinkiang on October 14, 1970 and the series of air explosions from nuclear tests at the USSR test site at Novaya Zemlya (74°N, 150°E).

The Tunguska sound waves were of acoustic modes S_0 and S_1 and travelled at group velocities between 280 and 310 m s^{-1} . The seismic event was excited by the air explosion at the source, energy being radiated outward from the epicentre by a series of wave motions. The Rayleigh mode, a rolling motion of the Earth's surface, moved at about 2.7 to 3.5 km s^{-1} . SH body waves, where the displacements are horizontal and perpendicular to the direction of propagation were recorded only at Irkutsk and the time of their arrival, coupled with arrival times of other waves at other observatories gives, according to Ben-Menahem, the explosion time to be 00 h 14 m 28 s UT. This time differs by about 30 s from previous estimates. The time delay between the arrival of the SH wave and the Rayleigh wave indicates that the main explosion occurred at a height of 8.5 km above the ground. The direction and extent of fallen trees around the epicentre suggest that this phenomenon was caused by the superposition of two waves—a spherical shock wave (due to the explosion, equivalent to a vertical point impulse of 7×10^{18} dyne s) and a conical ballistic wave (due to the moving incident object, equivalent to a horizontal point impulse of 1.4×10^{18} dyne s). The extent of the damage and the magnitude of the acoustic and seismic waves

indicate that the explosion had an energy of $(5 \pm 1) \times 10^{23}$ ergs, equivalent to 12.5 ± 2.5 megatons of TNT.

Ben-Menahem does not leap into the controversy as to the actual form of the incident object simply referring to it as a "UFO". Jackson and Ryan (*Nature*, **245**, 88; 1973) thought it might have been a black hole with the mass of a large asteroid (10^{20} – 10^{22} g) and a negligible geometrical radius. This would have shot straight through the Earth but unfortunately for the theory (although fortunately for us) the exit point, latitude, 40–50°N longitude 30–40°W, in the mid-Atlantic was not marked by an equally severe shock and blast wave. Hunt, Palmer and Penny (*Phil. Trans. R. Soc. Lond.*, **A252**, 275; 1960) consider the possibility of an extraterrestrial almost-critical mass of fissionable material becoming tamped on entering the atmosphere. It is difficult, however, to conceive how a sufficient amount of, say, deuterium and tritium can be compressed and heated to several million degrees centigrade and maintained in that state sufficiently long for the self heating to carry the reaction to the explosive stage merely by entry into the Earth's atmosphere. Cowan, Athuri and Libby (*Nature*, **206**, 861; 1965) suggest that an incident anti-matter body annihilated itself in the atmosphere, but no large anti-matter bodies have, as yet, been observed and it is hard to understand how it penetrated to such a depth in the atmosphere and why the explosion maximised at the end of the trajectory and not midway along it.

The most likely theory, that a small comet struck the Earth, was put forward by Whipple (*Q. Jl R. Meteorol. Soc.*, **56**, 287; 1930) and Astapovich (*Astr. J.*, **10**, 465; 1933). The trajectory of the bolide indicated that the comet was travelling in the opposite direction to the Earth and that the head-on collision was at a velocity of 60 km s^{-1} . The cometary nucleus, which, according to Whipple, is a large dirty snowball—a loose collection of dust and rock interspersed with water, methane and ammonia ices—exploded above the Earth's surface. If most of the energy came from the dissipation of kinetic energy and only a negligible portion from the chemically explosive reactions between the air and the radicals in the nucleus then the mass of the incident comet can be calculated to be approximately 3×10^{10} g. The fact that this comet nucleus has a diameter of about 40 m, more than an order of magnitude below the diameters estimated for visual comets, explains why it wasn't seen as it approached on its collision course to the Earth.

Further evidence favouring this hypo-

thesis is that the night sky luminescence after the fall was only observed over Siberia, Russia and Western Europe. Calculations of the comet orbit indicate that the dust and gas tail, which is directed away from the Sun, extended in a north-westerly direction from the point of impact. The dissipation of this tail in the atmosphere increased the night sky brightness by about 50–100 times. The discovery of many small (5–450 μm diameter) magnetite and silicate globules in soil samples taken from the Tunguska site also supports the comet hypothesis. These were formed when molten 'rain drops' of dust solidified as they slowly drifted to Earth after the explosion of the retarded nucleus. Flux estimates indicate that a cometary nucleus of this size will hit the Earth about every 2,000 yr, the rarity of the event giving ample justification for visiting Tunguska yet again.

Far-infrared balloon astronomy

from a Correspondent

THE far-infrared region of the electromagnetic spectrum—wavelengths between 20 μm and 1 mm—is probably the last regime to be explored by the astronomer. The reason for this apparent lack of resolve is not that the wavelength range is uninteresting—far from it when we realise that the majority of the many clouds of dust scattered throughout our Galaxy radiate most of their energy at precisely these wavelengths. The problems are technological; in the far-infrared, the Earth's atmosphere is virtually opaque to external radiation because of severe attenuation by water vapour molecules. There are very poor transmission windows at wavelengths of 350 μm , 450 μm , and around 800 μm , but these are accessible only from very high, dry sites such as Mauna Kea at 14,000 feet in Hawaii. Even then, transmission figures better than 30% are very rare. The obvious answer is to observe from above the absorbing layers in the atmosphere, and with dramatic improvements in balloon astronomy, many exciting discoveries are being made.

The University College London (UCL) group, led by R. E. Jennings, has pioneered balloon far-infrared astronomy and its latest flights have produced a catalogue of far-infrared sources in the Galactic plane. The 40-cm telescope, operating at an altitude of about 100,000 feet is directed to raster scan selected regions of the Milky Way, most of these having

been chosen because they are known from radio observations to contain complex H II regions.

These ionised gas domains surrounding a recently formed, high luminosity star are most intriguing astronomical objects. The unravelling of the detailed mechanisms of these star formation centres occupies a considerable fraction of astronomical effort and the problems are many and complex. The cores of these regions are thick with dust, often rendering optical identification of the ionising star or protostar extremely difficult, if not impossible. Near-infrared observations however do often reveal stars and protostars surrounded by very hot dust shells whose signals can be the free-free radio continuum emission of the H II region. These regions are also rich sites of molecular clouds, and emission maps of many and various molecules have been obtained at wavelengths of a few millimetres or centimetres for the most intense sources. To attempt to piece together the energetics of the emission nebulae, one must determine the total luminosities of the gas, dust and molecules and so derive the luminosity of the exciting star or stars, which must be the powerhouse of the whole region.

The key to understanding these regions is the presence of dust. The dust grains may be associated with the Stromgren sphere of the H II region and in this case are being heated by the direct flux of stellar ultraviolet radiation or by Lyman α radiation of the hydrogen gas surrounding the star. Alternatively, the grains could be associated with the dense molecular clouds found in and around H II regions, the grain heating being caused by stellar or protostellar objects within these molecular clouds. In the former case, the dust emission, peaking at say 100 μm , should be centred on the position of free-free continuum emission, whereas in the latter, the far-infrared emission should follow the contours of the molecular emission.

Using the UCL balloon system, operating in the wavelength range 40 μm to 350 μm , Furniss *et al.* (*Astrophys. J.*, **202**, 400; 1975) have produced the most detailed far-infrared map of the complex region W3 currently available. Their map reveals that the far-infrared emission seems to follow the radio emission in general, although there does appear to be a long spur projecting westwards from the main source. The authors claim that recent radio free-free emission has also been reported to possess this spur feature and so the tie-in with the radio emission rather than the molecular cloud emission would seem to be more favoured for this source at least.

This, however, is only one source and there are many others; the Great

Nebula region of M42 in Orion, for instance, one of the most exciting and complex regions in the sky, shows many differences to W3. It is also found that as one observes at longer wavelengths ($\sim 1\text{ mm}$) one is looking at the cooler dust and this component does appear to follow the molecular cloud emission rather than the H II continuum free-free emission. Once again the complexities of the regions are daunting and challenging. Theoretically one is not at all sure what size are the dust grains in the regions, or of what material; silicates or graphites are the usual choice. Laboratory spectral analysis of selected grain compositions is urgently required by the grain theoretician. Observationally one requires better high resolution, far-infrared and molecular maps and a near-infrared search for the dust-enshrouded protostars in the H II regions before the vastly complex physics and mechanics of these birthplaces of stars can be understood fully. In the far-infrared, one looks to the future for balloon and aircraft-based spectrometers and interferometers. The larger diameter balloon-based telescopes and the first non-military, infrared satellite, scheduled for launch in 1981, will open up a new era of observing in this wavelength range. $\square$

System for steroids

from Robert Shields

An understanding of the way in which steroid hormones regulate gene expression has been frustrated continuously by the lack of suitable model systems in which hormone action can be studied *in vitro*. Ideally what is required are hormonally responsive cell lines where mutant cells can be isolated and the mechanism of hormone action can then be dissected in the way that proved so successful in prokaryotes. Several steroid responsive cell lines are now available, including some potentially useful mutants (Sibley and Tomkins, *Cell* **2**, 213; 1974) but they all have a number of drawbacks. Now it looks as if the endocrinologists' prayer is about to be answered, for not only is an almost ideal system available, but combining as it does both endocrinology and tumour virology it should hit the double grant jackpot.

Recently it has been shown that physiological concentrations of glucocorticoids can stimulate the production of mouse mammary tumour virus (MMTV) in a number of cell lines established from mouse mammary adenocarcinomas (Parks *et al.*, *Science*, **184**, 158; 1974). This increase in virus production results from increases in virus specific RNA transcribed from the DNA provirus integrated in the host cell genome (Ringold *et al.*,

Virology, **65**, 135; 1975; Parks *et al.*, *J. biol. Chem.*, **250**, 3330; 1975). Induction of this viral RNA is mediated through the classical steroid-receptor mechanism since binding of various glucocorticoids to the receptor and transfer of the receptor-bound steroid to the cell nucleus are correlated with the increase in virus production (Young *et al.*, *J. biol. Chem.*, **250**, 3337; 1975; Ringold *et al.*, *Cell*, **6**, 299; 1975). Whether the increases in virus specific RNA produced by glucocorticoids involves a decrease in RNA degradation or an increase in transcription (which seems more likely) is not yet known. This is a vital point since transcriptional compared with post-transcriptional control has been a contentious point in steroid action for some time (see News and Views, *Nature*, **258**, 477; 1975). In any event the action of the steroid appears to be direct since inhibitors of protein and DNA synthesis do not block increases in viral RNA, no general increase in RNA synthesis is seen, and the concentration of the virus specific RNA doubles within 30 min of steroid addition. This effect of glucocorticoids is not confined to B-type RNA tumour viruses (such as MMTV). Glucocorticoids also increase C-type RNA virus produced in cells treated with halogenated pyrimidines (Paran *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2391; 1973) and enhance the production of polyoma (DNA) virus from productively infected mouse embryo cells (Morhenn *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1088; 1973).

What is particularly interesting about these systems is that steroids appear to be unable to initiate the transcription of virus sequences, and merely enhance the production of viral RNAs that are produced constitutively. Hence synthesis of C-type virus in KA31 non-producer cells is not induced by glucocorticoids but if virus is first induced with IUdr, virus production is increased many-fold by the hormone. Nor is constitutive production of viral RNA sufficient for a hormone effect since in murine epithelial cell lines constitutively transcribing both B-type and C-type virus sequences only the B-type transcripts are increased (Parks *loc.cit.*). Thus it may generally prove to be that steroids are only gene amplifiers and not true inducers.

Glucocorticoid control over virus production is a particularly good model system because viral RNA can be quantitated and tumour virologists have all the tools necessary to characterise mutant cells defective in various stages of viral production. In the not too distant future it may prove possible to remove the DNA provirus from the cell, together with its steroid responsive control elements. $\square$

articles

X-ray studies of linear polyiodide chains in α -cyclodextrin channels and a model for the starch-iodine complex

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The crystal structures of complexes of α -cyclodextrin (cyclohexaamylose) with Li^+ - and Cd^{2+} -polyiodide have been analysed by single crystal X-ray methods. The molecules are arranged head to head and linked by hydrogen bonds yielding endless stacks with channel cavities of about 5 Å diameter in which the iodine chains are located. In the Li^+ complex, the polyiodide chain is slightly zigzagged.

LINEAR polyiodine and polyiodide chains included in organic matrices are interesting for their unusual physical properties. The best known examples in analytical chemistry are the "blue starch-iodine" reaction, and in optics the polarisation filters based on complexes between polyiodide and organic polymers¹. The nature of the blue or black colour of these compounds has been studied spectrophotometrically² and explained by the electron gas theory³. The one-dimensional metallic properties, such as anisotropic conductivity and weak paramagnetism⁴, of polyiodide and polyiodine⁵ chains have been pointed out. As no detailed picture of an isolated linear polyiodide chain⁶ had been developed, it seemed necessary to study the α -cyclodextrin-polyiodide complex which preliminary studies have indicated contain well defined polyiodide chains^{3,7}. (Reddy *et al.*⁸ found linear chains of polymerised $(\text{I}_3^-)_n$ units in the HI_3 benzamide complex. These chains, however, occur in pairs which are stabilised by proton bridges.)

α -Cyclodextrin (cyclohexaamylose, α -CD) is obtained through degradation of starch by *Bacillus macerans* amylase. It consists of six α (1 $\rightarrow$ 4) linked glucoses (Fig. 1) and forms a truncated cone with height 8 Å, diameter 14 Å and an annular aperture of 5 Å. Owing to this aperture α -CD can form inclusion complexes with small molecules^{3,7} and these complexes crystallise in cage or channel structures, depending on whether the α -CD molecules are arranged crosswise or stacked on top of each other like coins in a roll⁸. The channel type structures bear some relationship to the helical starch molecules for the α -CD molecules form a hollow cylinder, with a geometry similar to that deduced for starch⁹.

When α -CD is crystallised from aqueous solution in the presence of iodine, cage type crystal structures with isolated iodine molecules are formed¹⁰. When metal iodides are added to the solution, channel type structures with polyiodide chains develop, exhibiting either triclinic, tetragonal or hexagonal space group symmetries, depending specifically on the added cation (W. S., U. Bergmann, M. N. and P. C. Manor, unpublished). This article describes the crystal structures of the

complex $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$ (triclinic) and $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I}_2 \cdot 26\text{H}_2\text{O}$ (tetragonal).

Polyiodide chain in $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$

Table 1 shows data for the triclinic, pseudo-hexagonal crystals of $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$. The heavy atom structure has been solved using tangent formula refinement of the rough model obtained from a Patterson distribution while the α -CD atoms, the water molecules and the cation had to be located from difference Fourier syntheses. The structure was refined by full matrix least squares cycles to a discrepancy index $R = 8.8\%$.

The crystal structure of the complex is determined by hexagonally packed α -CD stacks parallel to the crystallographic c axis with unit cell repeat distance 15.88 Å (Fig. 2a). In these stacks, the α -CD molecules are tilted by about 7° against the

Fig. 1 Chemical structure and nomenclature of α -CD.

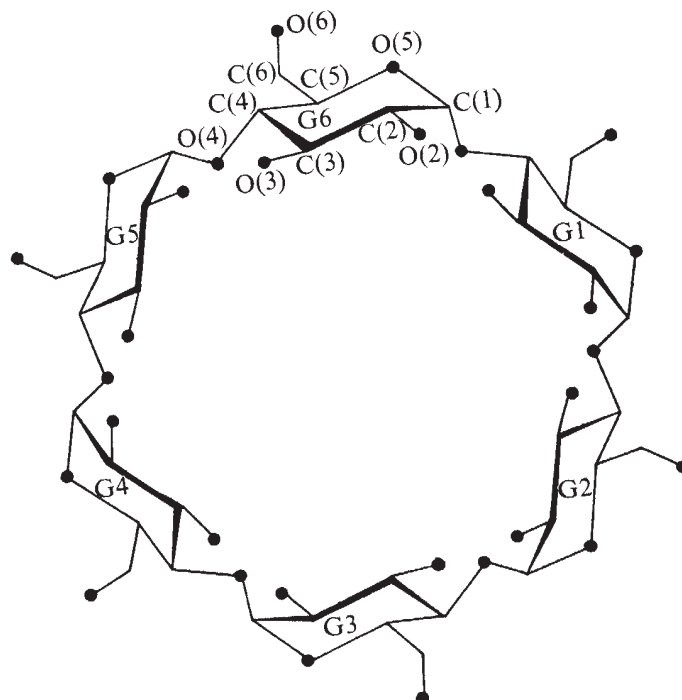


Table 1 Crystallographic data

Complex	$(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$	$(\alpha\text{-CD})_2 \text{Cd}_{1/2} \cdot \text{I}_2 \cdot 2\text{I}_2 \cdot 26\text{H}_2\text{O}$
Habitus	Hexagonal plates or needles	Needles with rectangular cross section
Colour	Black-brownish, metallic lustre	Black-greenish, metallic lustre
Space group	Strong dichroism	Strong dichroism
Cell constants	P1 $a = 13.88 \text{ \AA}^*$ $b = 13.88 \text{ \AA}$ $c = 15.69 \text{ \AA}$ $\alpha = 94.1^\circ$ $\beta = 87.8^\circ$ $\gamma = 119.9^\circ$	$P4_22_12$ $a = b = 19.93 \text{ \AA}^*$ $c = 30.87 \text{ \AA}$
Density calculated	1.72 g cm ⁻³	1.69 g cm ⁻³
Density measured	1.72 g cm ⁻³	1.70 g cm ⁻³
X-ray data collected	5,600	3,400
Final R factor	8.8%	12.0%

* a (tetragonal) $\approx \sqrt{2} \cdot a$ (triclinic); c (tetragonal) $\approx 2 \cdot c$ (triclinic)

stack axis, thereby prohibiting a truly hexagonal space group. The α -CD molecules are arranged head to head to form dimers which include five iodine atoms (Fig. 3) and are linked along the stacks through intermolecular O(2) $\cdots$ O(3) and O(6) $\cdots$ O(6) hydrogen bonds and through bonds to the eight water molecules located in the 'empty' space between the stacks. Further, the Li^+ cations are coordinated to O(2) and O(3) hydroxyl groups of α -CD molecules joined in dimers and to one water molecule, the ligands forming a distorted tetragonal pyramid; the shortest $\text{Li}^+ \cdots \text{I}$ distance, 5.05 $\text{\AA}$ exceeds ion-ion contact of 2.88 $\text{\AA}$.

The individual α -CD molecules display almost hexagonal symmetry. The glucoses are in the C1 chair conformation with all C(6)-O(6) bonds in the preferred *gauche*, *gauche* position¹¹, that is they are pointing away from the α -CD molecular centre (Fig. 3). The wide sides of the α -CD cones are lined by the O(2) and O(3) hydroxyl groups which form a ring of intramolecular hydrogen bonds between adjacent glucoses with O $\cdots$ O distances in the range 2.78–3.03 $\text{\AA}$.

The arrangement of the iodine atoms in the polyiodide chain follows the inclination of the α -CD molecules towards the stack axis (Fig. 3). In an α -CD dimer, the positions of four iodine atoms lying in the narrow α -CD cavities are fully occupied but the iodine atom near the wide O(2), O(3) rim of the α -CD torus is twofold disordered at 70%: 30% occupancies. Since the two disordered iodine sites are only 1.7 $\text{\AA}$ away from each other they cannot be occupied simultaneously and at all events clearly belong to one of the neighbouring I_2 units; therefore, alternating I_2 and I_3^- units can be distinguished.

As often observed, the I_3^- units are not strictly linear¹² but bent to about 170° and the two I-I distances are not equal to each other. In the I_2 units, the standard I-I distance of 2.67 $\text{\AA}$ is lengthened to 2.81 and 2.87 $\text{\AA}$ and the van der Waals' separation between iodine atoms of 4.3 $\text{\AA}$ is shortened to at least 4.03 $\text{\AA}$ (Fig. 3).

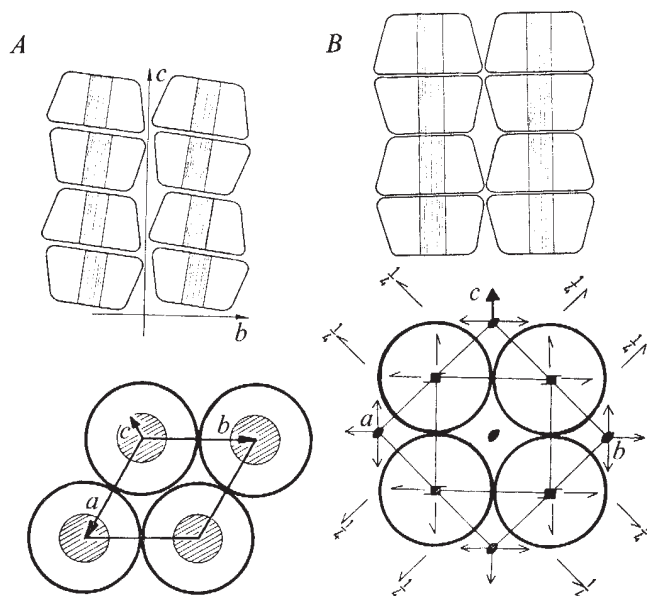
Polyiodide chain in $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I}_2 \cdot 2\text{I}_2 \cdot 26\text{H}_2\text{O}$

The $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I}_2 \cdot 2\text{I}_2 \cdot 26\text{H}_2\text{O}$ complex crystallises in the high symmetry space group $P4_22_12$. The heavy atoms were located by direct methods but the α -CD molecule could only be found by trial and error. The cation and water molecules were located from difference Fourier syntheses and the atomic parameters were refined to $R = 12.0\%$. Two half, independent α -CD molecules constitute the asymmetric unit and are related to the other two halves by the diad component in the 4_2 operation (Figs 2b and 4). Thus the α -CD molecular axes coincide with the 4_2 axis, the two α -CD molecules again being arranged as dimers in a head to head fashion, but the intermolecular O(2) $\cdots$ O(3) and O(6) $\cdots$ O(6) hydrogen bonding pattern is not the same because the α -CD molecules are rotated differently against each other (Figs 3 and 4). The dimers form stacks which are only loosely packed owing to the

requirements of the tetragonal lattice (Fig. 2b) and the open space between the stacks is filled by 26 water molecules per α -CD dimer, some of them being statistically disordered. The Cd^{2+} cation itself is twofold disordered, with both sites located on the twofold axes. They are coordinated octahedrally only by water molecules and $> 9.97 \text{ \AA}$ from the polyiodide chain. The conformation of the α -CD molecules themselves is similar, as discussed above.

Four of the five iodine atoms in the dimer are located on the 4_2 axis and therefore they must be colinear. Similarly as in the $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$ complex, the iodine atom near the wide O(2), O(3) rim of the α -CD torus is twofold disordered in equal amounts, which leads again to angles near 170° in the polyiodide chain. The two sites are too close together (1.24 $\text{\AA}$) to allow simultaneous occupation (Fig. 4). In contrast to the separation into I_3^- and I_2 units in the Li^+ -complex, however, the distribution of the iodine atoms follows a pattern, $(\text{I}_2 \cdots \text{I}^- \cdots \text{I}_2)_n$, usually considered as I_5^- , with the iodide ion I^- being disordered. The distances between the central iodine ion and the next iodine molecules, 3.17 and 3.14 $\text{\AA}$, are comparable with previously observed I_5^- structures¹³. The separation of 3.32 $\text{\AA}$ between the I_5^- units is considerably shorter than the expected van der Waals' distance of 4.3 $\text{\AA}$. These

Fig. 2 The packing scheme of α -CD stacks in (A) the triclinic $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$ complex, (B) the tetragonal $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I}_2 \cdot 2\text{I}_2 \cdot 26\text{H}_2\text{O}$ complex. The unit cell constants (a) and (b) are determined by the dimensions of the closely packed α -CD molecules (see also Table 1).



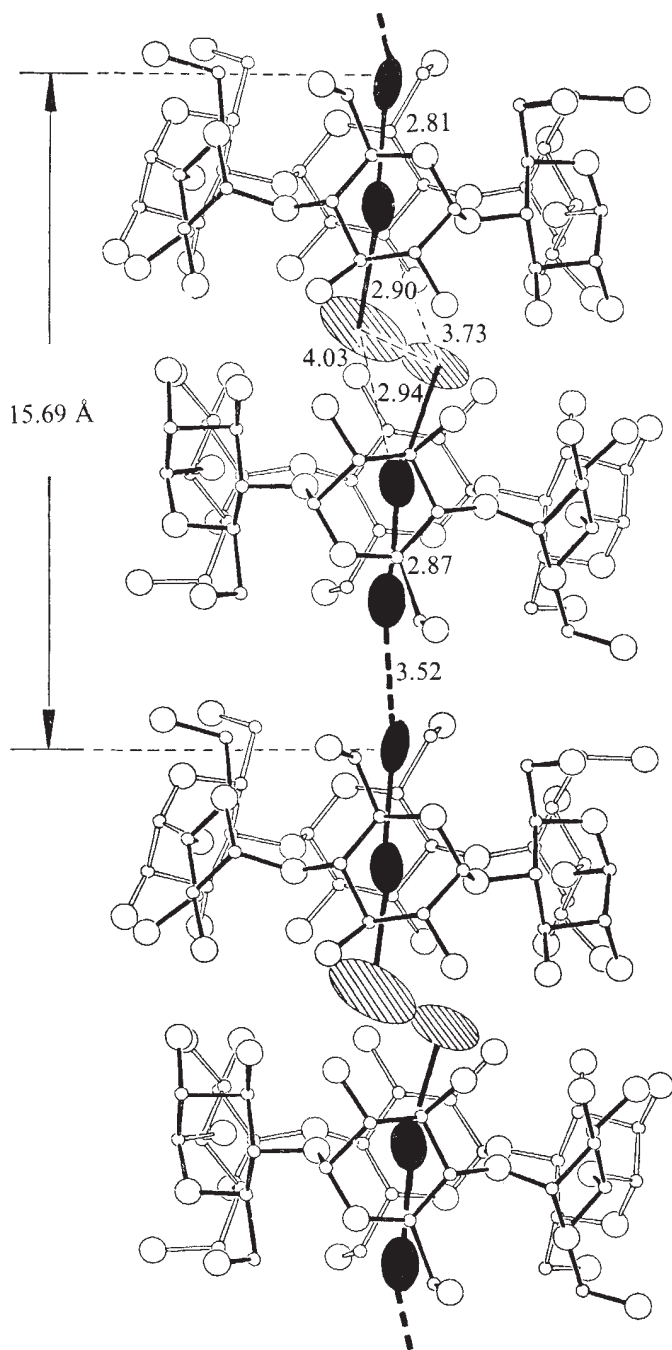


Fig. 3 The structure of the $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$ complex, projected on the ac plane, water molecules and Li^+ ion not drawn. o, C; $\circ$, O; fully and statistically occupied iodine sites are indicated by filled and hatched ellipses representing the anisotropic thermal vibration of these atoms. Distances between iodine atoms are given in Å units. The crystallographic asymmetric unit is marked by dashed lines.

results indicate that in this polyiodide chain extensive delocalisation of electrons occurs.

Conductivity measurements

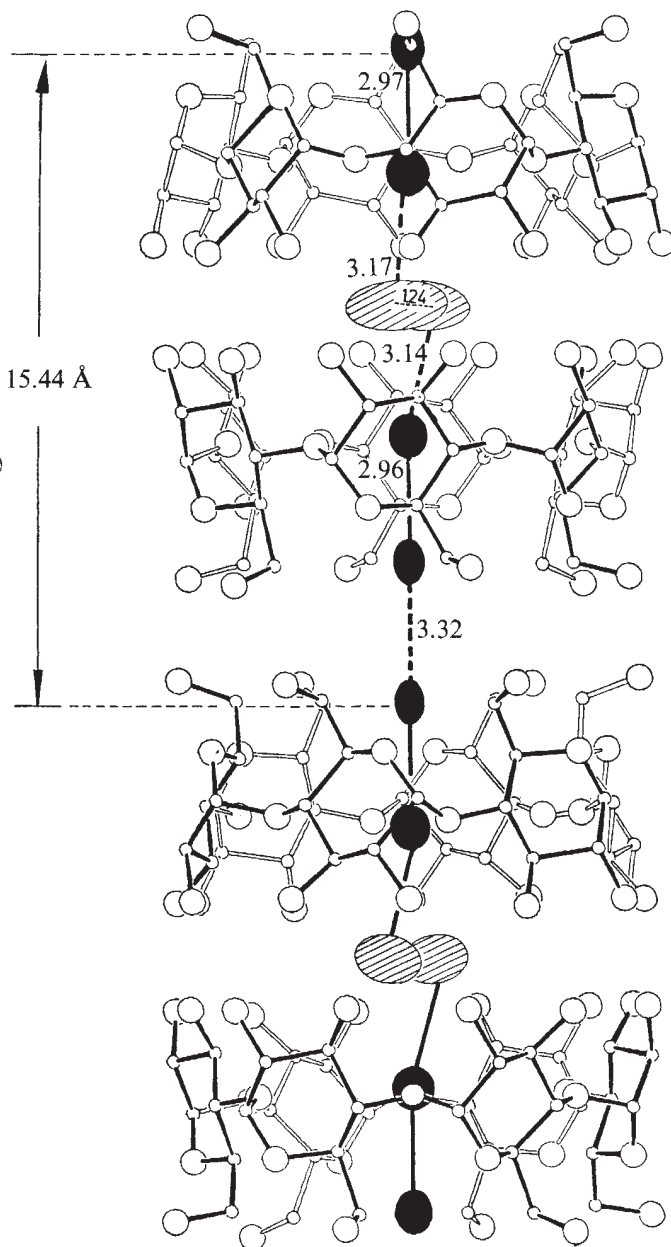
Several complexes of polyiodide with organic molecules have been investigated by X-ray crystallography¹³⁻¹⁵. In these structures the polyiodide chains consist of clearly distinguishable I_2 and I_3^- units in a zigzag arrangement. Linear, isolated polyiodide chains⁶ have been observed in detail only in the two crystal structures described above and in the trimesic acid

polyiodide¹⁵ complex. Although in the two $\alpha\text{-CD}$ complexes the iodine and $\alpha\text{-CD}$ atoms were located and refined on the basis of three-dimensional X-ray data, the structure of the polyiodide chain in the trimesic acid complex was derived from one dimensional diffuse layer lines.

In the trimesic acid polyiodide complex, the periodicity along the polyiodide chain is also about 15.5 Å, five iodine atoms are arranged as centrosymmetric ($\text{I}_2 \cdots \text{I} \cdots \text{I}_2$) units with I-I distances of 3.26 Å, 2.74 Å and 3.50 Å¹⁵, in close agreement with the distances observed for the tetragonal complex described above: 3.14 (3.17), 2.96 (2.97) and 3.32 Å. In the latter the tendency to achieve equidistant spacings is more obvious, and extensive delocalisation of electrons should be expected.

For that reason, preliminary conductivity measurements were carried out in the direction of the polyiodide chain of the $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I} \cdot 2\text{I}_2 \cdot 26\text{H}_2\text{O}$ complex. The conductivity was about $10^{-6} \Omega^{-1} \text{cm}^{-1}$, in agreement with results obtained previously with the same complex (M. M. Labes, personal

Fig. 4 The structure of the $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I} \cdot 2\text{I}_2 \cdot 26\text{H}_2\text{O}$ complex. For further details, see legend to Fig. 3.



communication), and suggests that the polyiodide chain has the properties of a semiconductor. In summary, in strictly linear polyiodide chains I_5^- units with a fine structure ($I_2 \cdots I \cdots I_2$)⁻ seem to be preferred but in nonlinear, slightly zigzagged polyiodide chains I_3 and I_5^- units can be distinguished. In complexes between polyiodide and polymer organic molecules such as starch or the polarisation filter polymers there are no crystallographic restrictions and it is to be assumed that slightly zigzagged chains will occur. It is an outstanding feature of the α -CD-polyiodine complex that the cations are located outside the α -CD channels at distances well beyond the expected $Li^+ \cdots I^-$ or $Cd^{2+} \cdots I^-$ ion pair interaction, yielding a negatively charged polyiodide embedded in the insulating organic material α -CD. In other structures, the tendency to form ion pairs with polyiodide is obvious^{6,14}.

Intermolecular forces

Charge transfer interactions between iodine atoms and the ether-like O(4) linkages of α -CD molecules have been reported to stabilise the α -CD (or starch)-polyiodide complexes¹⁸. Our own and earlier¹⁰ X-ray crystal studies show that the main interactions between iodine atoms and α -CD molecules are van der Waals' contacts with the C(3)-H and C(5)-H methin hydrogen atoms: viewed from the iodine atom positions, the O(4) atoms are buried behind these hydrogen atoms and at least 0.68 Å further away than the $I \cdots O$ van der Waals'

contact of 3.55 Å. Therefore, distances in the range between covalent and van der Waals' which are demanded for charge transfer type interactions¹⁷ are not possible. As for the α -CD-polyiodide complexes, it is unlikely that the starch-polyiodide complexes are stabilised by $I \cdots O$ charge transfer interactions.

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The role of long range forces in ordered arrays of tobacco mosaic virus

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We examine the role of electrostatic repulsion and van der Waals' attraction in determining interval spacings in 'equilibrium' gels of tobacco mosaic virus. The observed spacings are inconsistent with a force-balance model. Experimental probes of the role of forces are suggested.

EVER since Bernal and Fankuchen's description, in 1941, of the parallel packing of tobacco mosaic virus (TMV) particles in the ordered phase of a two-phase system¹, the TMV system has been considered an ideal example of spontaneous self-organisation. Since some biological structures, such as muscle sarcomere^{2,3}, visual cornea⁴⁻⁸, and the gel of sickle cell haemoglobin (ref. 9 and A. P. Minton, personal communication), appear to be ordered arrays of long proteins or protein aggregates, one is tempted to make analogies between those structures and the array of the rod-like TMV particles. Possibly the observed spacings in TMV gels result from balancing the electrodynamic van der Waals' attraction, and electrostatic repulsion¹⁰⁻¹². Primarily because of limitations in previous physical theory, this assumption has never been adequately examined. Here we have used new methods of computation to explore the role of long range forces in the TMV system.

There has been a fairly clear but arbitrary dichotomy in theoretical analyses. First, it has been shown that without any attractive force between rod-like particles, steric interference between asymmetric bodies is sufficient to cause a separation of the rod population into an ordered phase of parallel rods in

equilibrium with another phase of randomly oriented rods in suspension^{13,14}. Second, work on long-range interactions has shown that energies of attraction can be comparable to the energy of Brownian (random) motion when bodies are close compared with their size¹⁵. Such interactions might therefore have a significant role in overcoming Brownian motion to align like particles.

The relative importance of these factors in the formation of the observed ordered arrays has remained an open question. The truncated virial expansion technique^{13,14} used to describe the consequences of steric repulsion is not very accurate at the high particle concentrations at which observations were made. Such a treatment predicts transition densities, but cannot provide unambiguous values for the rod-rod spacing. The alternative lattice models are also not designed to predict observed spacings (ref. 16, and A. P. Minton, personal communication). Although experimental examination of uncharged polypeptides in organic solvents¹⁷ shows that ordering of very long rods arises primarily from steric repulsion, none of the available hard rod models is able to account for the observed transition densities in TMV suspensions¹⁸.

In the following, we compare observed interval separations with those predicted by force-balance. We use the theory of balancing forces to derive a constraint between separations and the salt concentrations in the medium, and we use recently published absorption spectra to make a numerical calculation of the van der Waals' attraction between proteins in salt water.

Two conclusions are immediately apparent: first, the magnitudes of the observed spacings are always much greater than

those expected from the positions of the energy minima. Second, the observed change in separation on varying the salt concentration in the medium cannot be fitted by shifts in the positions of equilibrium. (This is true even if one chooses unphysical coefficients for the competing forces.)

We suggest experiments for more critical examination of the role of forces, including repetition of the early spacing measurements¹ in more stringently defined conditions of pH and salt concentration in the medium.

Force-balance constraint between spacing and salt concentration

The leading term in the electrostatic repulsive force per unit length between parallel rods is¹⁹

$$f^{es} = \frac{2v^2e^2}{\epsilon a} \left(\frac{2}{\pi}\right)^{1/2} \frac{\exp[-\kappa(R-2a)]}{\kappa R} \quad (1)$$

Equation (1) is valid when the rod length is much greater than the separation of the rods. R is the interaxial distance, a the rod radius, v the net number of unit ionic charges per unit length of the rod, assumed to be smeared uniformly on the outermost surfaces. The Debye constant κ is

$$\kappa^2 = \frac{8\pi ne^2}{\epsilon kT}, \quad n = \frac{1}{2} \sum_{\{n_i^0\}} n_i^0 z_i^2 \quad (2)$$

with $(-e)$ the electronic charge, ϵ the dielectric constant of the medium, which is assumed uniform, k the Boltzmann constant, T the absolute temperature, and the n_i^0 are the concentrations of ions of valence z_i in the bathing medium far from the rods. Equation (1) assumes that the radius a is greater than the Debye length κ^{-1} , and that the electrostatic energy of a counterion near the rod is smaller than the thermal energy kT . Expressions more general than equation (1) have been derived and may be applied if needed^{10,19}.

The electrodynamic attractive force per unit length between the same two rods has the approximate form^{10,20}

$$f^{ed} = -\frac{2\pi A_H}{3R^2} S \quad (3)$$

where S is the double sum

$$S = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{[(2m+2n+1)!!]^2 (2m+2n+1)}{m!n!(m-1)!(n-1)!} \left(\frac{a}{2R}\right)^{2m+2n} \quad (4)$$

(The double factorial is the product $q!! = q(q-2)(q-4) \dots$ (1 or 2).) Equation (3) neglects the retardation damping of van der Waals' forces. It also assumes that the dependence of the force on distance is obtained by pairwise summation of inverse sixth power interactions between incremental parts of the rods,

and that the rods are of uniform composition (although the formula may be very simply generalised to describe rods composed of concentric cylindrical layers, as might be appropriate for TMV particles). The magnitude of the coefficient A_H can be obtained from electromagnetic spectra of protein and water and will be computed below. 'Many-body' effects which might give a different form to f^{ed} will be negligible because of ionic screening of the low-frequency charge fluctuation forces most likely to demonstrate these effects. Again, more general formulae exist²⁰ and may be used if necessary.

If the interaxial spacings R observed by X-ray diffraction are the result solely of balancing electrostatic repulsion f^{es} and electrodynamic attraction f^{ed} , then these forces must sum to zero at the observed spacings R . Setting $f^{es} = -f^{ed}$ and sequestering coefficients, we have

$$F(R, \kappa) = \frac{S/R^2}{\exp[-\kappa(R-2a)]/\sqrt{(\kappa R)}} \quad (5)$$

$$= \frac{v^2e^2}{A_H} \left(\frac{2}{\pi}\right)^{1/2} \frac{3}{\pi\epsilon a}.$$

That is, $F(R, \kappa)$ is a constant for all predicted pairs of values of R and κ . The equation

$$F(R, \kappa) = \text{constant} = C$$

provides a constraint between spacing and salt concentration depending on the value of the constant C where

$$C = \left(\frac{v^2}{A_H}\right) \left(\frac{2}{\pi}\right)^{1/2} \left(\frac{3e^2}{\pi\epsilon a}\right) \quad (6)$$

Computation

We can compare the behaviour predicted in equation (5) with the available data on the TMV 'equilibrium gels'. Bernal and Fankuchen¹ provide a table of interaxis spacings as functions of salt concentrations; their results are presented in columns 1 and 2 of Table 1. The Debye constant corresponding to the various salt concentrations is given in column 3. The function $F(R, \kappa)$ defined in equation (5) is then tabulated in the fourth column. According to the arguments given in the last section, $F(R, \kappa)$ should be a constant if the spacing R corresponds to an energy minimum. This is not the case.

The criterion $F(R, \kappa) = C$ is a very sensitive test of the force-balance picture. This can be seen in Fig. 1, where the values of R and κ satisfying $F(R, \kappa) = C$ are plotted for $C = 0.01, 0.1, 5, 50$, and $100 \text{ \AA}^{-2}$; the experimental points are included as well. Note that there are very few experimental points, and that a relatively small error in spacing suggests a large variation in C . In fact, any value of C between 5 and 100 seems to be a reasonable choice based on available data. Unreported experimental error might be one cause of the irregular behaviour of the points plotted on Fig. 1.

The points at 186 and 182 Å correspond to surface-surface distances that are much too small for the present theory to be quantitative. We have chosen a rod radius of 90 Å for the calculations. The TMV particle is a composite structure built of subunits some 25 Å apart at the viral surface²¹. These subunits protrude from the viral rod, and units from adjacent parallel rods can dovetail by 30 Å (the closest axis-axis distance is 150 Å for dry preparations, while accurate X-ray studies²¹ on the wet particle suggest a particle diameter of 180 Å). The minimum interaxial distance of 173 Å observed by Bernal and Fankuchen is probably accurate, but arises from a 7 Å dovetailing of the viral subunits. Given the graininess of the viral

Table 1

$\bar{R}^*$ (Å)	Normality* equivalents (l ⁻¹)	κ (Å ⁻¹)	$F(R, \kappa)^\dagger$ (Å ⁻²)
251-302	0.26	0.2054	58.2-38.9 × 10 ⁴
229	0.33	0.2313	6.9
223	0.52	0.2904	35.4
210	1.03	0.4077	86.3
186	2.06	0.5780	0.583
182	4.12	0.8174	0.324

* Experimental values for interaxis spacing R and normality from Table III of ref. 1, salt = (NH₄)₂SO₄.

† See equation (5).

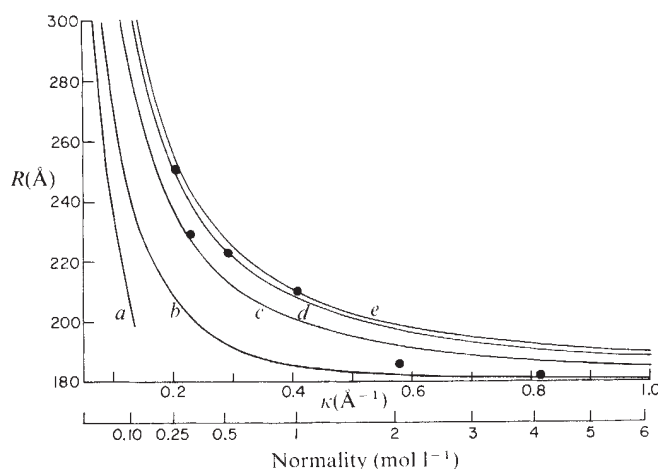


Fig. 1 The position of force-balance as a function of salt concentration in the bathing medium is plotted here for five values of the constant $F(R, \kappa) = C$. *a*, 0.01 Å⁻²; *b*, 0.1; *c*, 5; *d*, 50; *e*, 100; ●, experiment. The experimental points of Bernal and Fankuchen¹ are included for illustrative purposes only, and not as evidence for the propriety of the force-balance picture. The curve for $C = 0.01$ Å⁻² ends abruptly at $R = 198$ Å because no energy minima are found at smaller separations (attraction is always greater than repulsion).

surface it is inappropriate to use equation (5), which assumes a smooth cylindrical surface, for separations < 200 Å.

The theory above assumes that the viral rods will interact in pairs, which is in accord with Coulombic repulsions in salt solutions where the density of the mobile ions exceed the volume density of charges fixed to large particles, that is, where the Debye length of the medium is less than distances between the large bodies. (In fact the salt concentrations used experimentally are probably too high for equation (1) to be used with confidence for the repulsion of two viral particles.) The assumption of pairwise rod-rod attraction conforms well with the criterion that the polarisability of the whole gel is linear in rod density²².

Attractive forces and energy minima

Is there any *a priori* estimate of the constant C using equation (5)? For computation of the repulsive force, we know that there are probably between one and two ionic charges per protein subunit (D. L. D. Caspar, personal communication). We have estimated a Hamaker coefficient in the context of the Lifshitz formalism²³⁻²⁷ using spectroscopic data for water at frequencies at up to 25 eV (refs 28-29), and for bovine serum albumin in the frequency range 2-82 eV (ref. 30). These data were fitted to the form of damped linear oscillators by the method of Knott and Shrager^{31,32}. A_H was then computed by numerical integration, using the theory of Dzyaloshinskii *et al.*²³⁻²⁷. We neglected retardation damping of the van der Waals' force and assumed complete screening³³⁻³⁶ of the low frequency fluctuations by salt. A_H was found to be between 5×10^{-14} erg and 9×10^{-14} erg depending on the accuracy of the optical data.

For a rod with 2,130 subunits per 3,000 Å length and a 90 Å radius²¹, the constant C from equation (7) will be roughly between 0.01 and 0.1 Å⁻². This range of values is lower than all values in Table 1 whatever the spacing and salt concentration. That is, inter-rod distances are always greater than those expected from force-balance. Note that C is independent of the length. This is helpful since viral length can be indeterminate because of end-to-end aggregation of viral particles³⁷. Since our theory does not apply to the experimental conditions of Bernal and Fankuchen, one cannot draw any firm conclusions. The erratic estimates of the 'constant' C in Table 1 fall, however, well outside the reasonably expected range.

It further seems that well depths (the interaction energy at the equilibrium separation) are not large, but only $\lesssim kT$ (ref. 10).

This may be compared to the guess of 50 kT proposed in early work on force balance in muscle¹¹. Inclusion of retardation screening of van der Waals' attraction would only weaken the depth of the expected energy minimum.

Discussion

We expect that attractive forces will not strongly influence the arrangement of rod-like proteins at interaxial spacings > 200 Å. They may be important in circumstances of high concentrations of weakly charged particles. It is now possible to delineate those circumstances more precisely, and to develop a better intuition about the organisation of proteins in experimental systems less accessible but more functional than TMV suspensions.

The most critical examination so far on long range forces in TMV gels is the 1953 study of Gerald Oster^{37,38}. Working in a regime of low virus concentrations (2.5% by weight), and very low salt concentrations (~ 5 mM), he observed that the addition of electrolyte destroys the gel even while screening repulsive forces. He concluded that repulsion is the dominant factor in ordering; that to within experimental accuracy the Onsager model gave accurate estimates of viral concentrations in both phases, and that attractive forces need not be considered until fairly high viral concentrations, where, in addition, there was almost no electric charge on the particles. In this regime a third, precipitated, iridescent phase has also been observed in TMV, the existence of which is sensitive to electrolyte in the medium³⁹.

Our theory supports Oster's view and predicts negligible attraction between cylindrical particles in dilute solution. Since the interesting cases for the packing of linear proteins in other systems occur at higher volume fractions and higher salt concentrations than in Oster's work, it would be worthwhile to extend his observations to this regime to check our theoretical argument.

On this basis, it is possible to describe the experimental situations which would be most amenable to direct analysis. Specifically, one should work with low salt concentrations (probably < 0.5 M), low net charge densities on the rods (near the isoelectric point, pH 3.3 (ref. 1, noting that there is zero net charge at pH 4.5 when salt is absent from the bathing medium (D. L. D. Caspar, personal communication)) at interval distances larger than the distance between viral subunits (21 Å, ref. 21), and using solutions of monovalent salts rather than divalent or mixed valence salts. In these conditions, one may measure mean interaxial distances R for rods in the ordered (gel) phase of a two-phase system. These should be measured as a function of salt concentration (or Debye constant κ) at all pH where a two-phase system exists.

The determination of phase diagrams for TMV in water at various pH and salt concentrations can facilitate theoretical study. The concentrations of protein in regions of two phases (ordered and disordered) need to be mapped as a function of pH, salt concentration and temperature.

The concentration of weakly charged viral particles in the dilute disordered phase can indicate the depth of any significant interparticle energy. The repulsion-only theory suggests that there will be of the order of one particle per $\pi^2 d/4$ volume where d and l are the effective particle diameter and length. Note that the diameter d is here an effective diameter, because of electrostatic repulsion, and l may be an effective length due to end-to-end aggregation^{37,39}. They will be greater than the physical dimensions of the virus and be sensitive to salt concentration. If there is a strong binding (a negative energy $-W$) between each particle with its neighbours in the ordered phase, the concentration in the dilute phase will go down as $\exp(-W/kT)$ and W will be sensitive to salt concentration. Since this argument is only approximate, the existence of W will not be demonstrated unless the dilute concentration is qualitatively less than predicted for $W = 0$.

On the theoretical side, it would be useful to know the properties of a system of rods having a sloping repulsive potential and

a weak attractive force. This might best be done as a perturbation of the pure hard-rod solution of Onsager since this model seems to include the dominant features of rod ordering¹⁸. Brenner *et al.*^{40,41} have made initial attempts at including a shallow square well potential. Quantitative estimates of rod spacing await a more refined statistical-mechanical theory.

A better explanation of ordering in TMV arrays is needed before reliable conclusions can be drawn on the questionable role of balancing forces among muscle proteins^{2,3,10-12,42-44} or among proteins from other cells. We do not believe that long range protein attraction is a strong determinant of order in either viral or cellular protein arrays.

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Reiteration frequency of the gene for tissue-specific histone H5 in the chicken genome

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Chicken erythroid cells contain a tissue specific histone known as H5 in addition to the five major histone species found in other organisms. The mRNA coding for this histone has been isolated by indirect immunoprecipitation from immature, non-dividing reticulocytes in which this is the only histone synthesised. The mRNA has been modified by the enzymatic addition of a 3' polyadenylic acid tract, and transcribed into complementary DNA (cDNA) using the RNA-dependent DNA-polymerase from avian myeloblastosis virus. Studies on the hybridisation of this cDNA indicate that the gene coding for the H5 histone is reiterated 10 times in the chicken genome.

ANALYSIS of the eukaryotic genome is difficult because of the complexity of the DNA and the presence of sequences which are repeated many times^{1,2}. Nevertheless the kinetics of reassociation of pure mRNA, or its complementary DNA (cDNA) back to the total DNA of the genome, can be used to calculate the reiteration frequency of specific gene sequences. Such hybridisations have shown that many mRNAs, including those for globin^{3,4}, silk fibroin⁵, and ovalbumin⁶, are transcribed from unique sequences in the DNA. It has been demonstrated that most of the mRNA in a variety of cells is transcribed from this 'unique' fraction of the DNA⁷⁻⁹. Two exceptions to this are the genes for feather keratins¹⁰, and histones^{11,12} which are both found in the 'moderately repetitive' DNA. The histone genes in sea urchin embryos are present as tandemly repeated blocks

reiterated some 400-1,000 times (species variation) in the genome^{11,13}. Cross hybridisation experiments indicate that the histone genes are also reiterated in the *Drosophila*¹⁴ and *Xenopus*¹¹ genomes. Recent results show, however, that these genes are only reiterated some 10-20-fold in the genome of man¹⁵ and mouse (quoted in ref. 15).

Histone gene reiteration frequencies have all been calculated from experiments in which highly labelled mRNA coding for all five histones was used as a probe. The nucleated red blood cells of birds, amphibians, and fish contain a sixth tissue-specific histone species known as H5 (ref. 16; the nomenclature is taken from reference 17). It has been suggested that this histone is involved in the final termination of transcription in these cells¹⁸. H5 is the only histone produced by the immature non-dividing reticulocyte¹⁹ and its synthesis is therefore independent of DNA synthesis, unlike that of the other histones^{20,21}. The mRNAs coding for these other histones are detectable only during S-phase and are rapidly turned over on cessation of DNA synthesis^{22,24}. H5 mRNA on the other hand must be quite stable in the absence of DNA synthesis and thus subject to quite different controls. Whatever the biological significance of this, from a practical viewpoint it enables us to isolate the mRNA for a single histone species²⁵. We report here the isolation of this mRNA, its transcription to produce cDNA, and the use of this cDNA to estimate the reiteration frequency of the gene coding for this highly unusual histone in the chicken genome.

Isolation of H5 mRNA

Although H5 is the only histone produced by the avian reticulocyte¹⁹, the main protein product of this cell

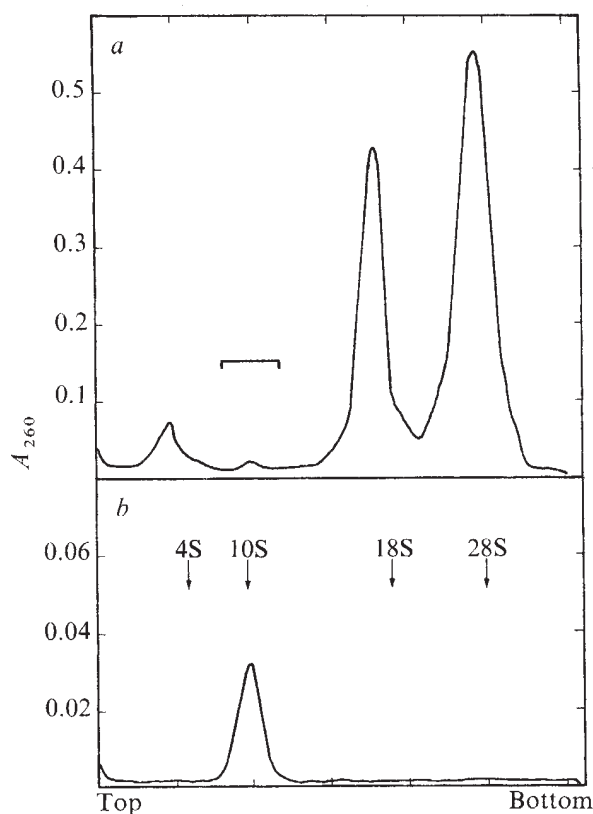


Fig. 1 *a*, Profile at 260 nm of sucrose gradient containing RNA from H5-synthesising polysomes. Chicken polysomes were prepared as described²⁵ and diluted to 25 A_{260} units ml^{-1} in 25 mM Tris (pH 7.6), 25 mM NaCl, 5 mM MgCl_2 , 1 $\mu\text{g ml}^{-1}$ Trichodermin (Leo Pharmaceutical Products), 0.1 mg ml^{-1} heparin. An aliquot (40 $\mu\text{g ml}^{-1}$) of rabbit anti-H5 (purified as described²⁵) was added and the mixture incubated at 0 °C for 1 h. The antibody-polysome complexes were then precipitated by the addition of purified goat anti-rabbit immunoglobulin (1 mg per 30 μg anti-H5). After 2 h at 0 °C (ref. 27) the precipitate was washed²⁸ to remove non specifically bound polysomes, and the RNA from the remaining polysomes was extracted with phenol²⁹. This RNA was disaggregated in formamide, diluted, and centrifuged through a 10–40% (w/v) sucrose–sodium dodecyl sulphate (SDS) gradient²⁵ in a Beckman SW41 centrifuge rotor at 40,000 r.p.m. for 16 h. The fraction indicated by the bar was pooled and further purified as below. *b*, Profile at 260 nm of sucrose gradient containing purified H5 mRNA. The RNA described above was purified by 2 cycles of oligo(dT)–cellulose chromatography³⁰, and the unbound RNA was then treated with formamide, diluted and centrifuged as above.

is globin²⁵. Furthermore, since the expected size of the H5 mRNA is very close to that of globin mRNA²⁵, it is impossible to use techniques based solely on the size of the mRNA to purify it. This has prompted the adoption of immunological methods to separate H5-producing polysomes. Indirect immunoprecipitation²⁶ is used to maximise the yield of H5 mRNA while minimising contamination with other mRNAs, especially globin mRNA. RNA was extracted from the polysomes²⁹, and the 8–12S RNA separated on sucrose gradients (Fig. 1*a*). Polyadenylic acid-containing RNA was removed by two passages through oligo(dT)–cellulose and the unbound H5 mRNA repurified by further sucrose gradient fractionation. The 10S RNA thus produced (Fig. 1*b*) did not act as a template for AMV RNA-dependent DNA-polymerase in conditions in which chicken globin mRNA was transcribed to 70% efficiency. This property, together with the lack of binding to oligo(dT)–cellulose, suggests that H5 mRNA is similar to other histone mRNAs in not having a 3' polyadenylic

acid tract^{31,32}. The H5 mRNA can be efficiently translated in the wheat embryo cell-free translation system to produce a single major product which coelectrophoreses with a ^{14}C -labelled H5 standard, on two different polyacrylamide gel systems (Fig. 2). The 10S mRNA thus contains very little translatable globin mRNA and the only mRNA detectable is that coding for H5. This does not exclude the possibility that 10S-sized non-mRNA contaminants are present in the RNA however.

Production and characterisation of H5 cDNA

In reactions which involve reassociation of trace amounts of a specific probe to a vast excess of total genomic DNA³⁶ it is essential that the probe is labelled to a high specific activity. This condition is fulfilled by the cDNAs produced from purified mRNA by AMV RNA-dependent DNA-polymerase, since highly labelled deoxynucleotide triphosphates are used as substrates³⁷. A further advantage is that such cDNA probes do not suffer from the instability problems associated with RNA probes³⁸. H5 mRNA was not, however, copied by the enzyme due to the absence of a 3' polyadenylic acid tract. This tract hybridises to short pieces of oligo(dT) which then act as a primer for the enzyme³⁹. A short stretch of adenylic acid residues was therefore added to the 3' end of the mRNA, by an ATP–polynucleotidyltransferase prepared from maize⁴⁰. The modified mRNA was then transcribed into cDNA and

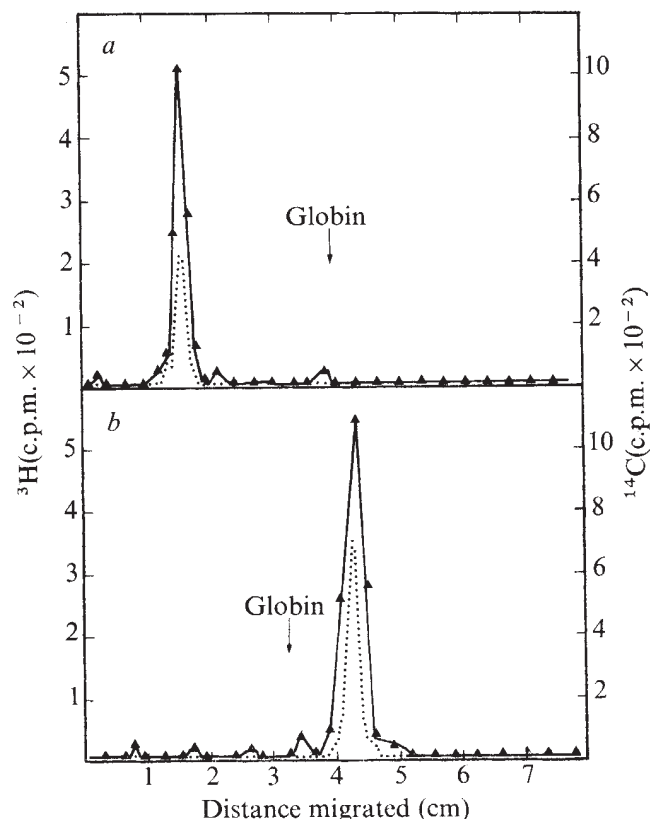


Fig. 2 Analysis on polyacrylamide gels of the translation products of H5 mRNA *in vitro*. H5 mRNA (0.5 μg) was translated in the wheat embryo cell-free system of Shih and Kaesberg³³ modified as described²⁵. The labelled cell-free products were electrophoresed on *a* the SDS–urea gels of Swank and Munkres³⁴ and *b* the low pH urea gels of Panyim and Chalkley³⁵ as modified¹⁹. The gels were then sliced and radioactivity measured as described²⁵. $\blacktriangle$ – $\blacktriangle$ ^3H -labelled product of translation *in vitro*; $\cdots$ ^{14}C -labelled H5 standard. Electrophoresis is from left to right in both cases. The ^{14}C counts accounted for 10% spillover into the ^3H channel and this was subtracted. The position of a ^{14}C globin standard is indicated.

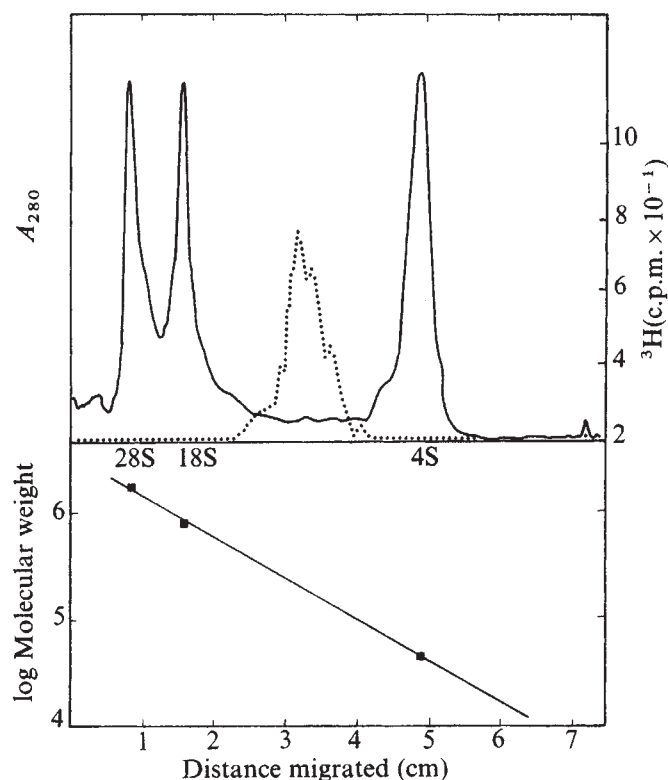


Fig. 3 Estimation of the size of the cDNA produced from polyadenylated H5 mRNA. H5 mRNA (1 μ g) was incubated at 30 °C for 3 h in a 30- μ l incubation mix containing 70 mM Tris-acetate (pH 8.6), 10 mM dithiothreitol, 1 mM ATP, 1 mM $MnCl_2$ and 50 μ g ml^{-1} of ATP-polynucleotidyltransferase. The modified RNA was then ethanol precipitated and cDNA was synthesised from it, in the same tube, as described¹⁰. A portion of the purified cDNA was then electrophoresed on 4% polyacrylamide gels containing formamide⁴⁷. 4S, 5S, 18S and 28S RNA were included as internal markers. Following electrophoresis the gels were scanned at 280 nm and then sliced and the pattern of radioactivity determined⁴⁸.

formamide gel analysis of the product indicated an average length of 450 nucleotides (Fig. 3).

Although little other mRNA translational activity was detectable in the H5 mRNA preparation (Fig. 2), 10S-sized ribosomal RNA (rRNA) breakdown products have been described³⁸. It is theoretically possible that the cDNA was preferentially copied from such contaminating RNA and it was therefore essential to prove that the cDNA made was complementary to H5 mRNA, and not to such contaminants. Since the two likely contaminating RNA species in the H5 mRNA preparation were rRNA and globin mRNA, this was readily tested by hybridising the cDNA to purified globin mRNA, 18S rRNA and 28S rRNA. The hybridisations were carried out in RNA excess and taken to a R_0t of 1 to ensure completion of the reaction. Hybrids were then assayed with S1 nuclease, a single-strand specific nuclease isolated from *Aspergillus*⁴¹. The data from such experiments allowed a direct estimate of the percentage of sequences in the cDNA preparation which were complementary to these different RNAs and are given in Table 1. The results show that H5 cDNA is contaminated only to about 3% with sequences complementary to rRNA (largely to 18S RNA) and a similar amount complementary to globin mRNA. If the globin mRNA used in these hybridisations had not, however, been purified by oligo(dT)-cellulose chromatography, then 85% of the H5 cDNA was protected from S1 degradation by the formation of hybrids (Table 1). This is merely indicative of the fact that chicken globin mRNA

Table 1 Extent of cross hybridisation of H5 cDNA

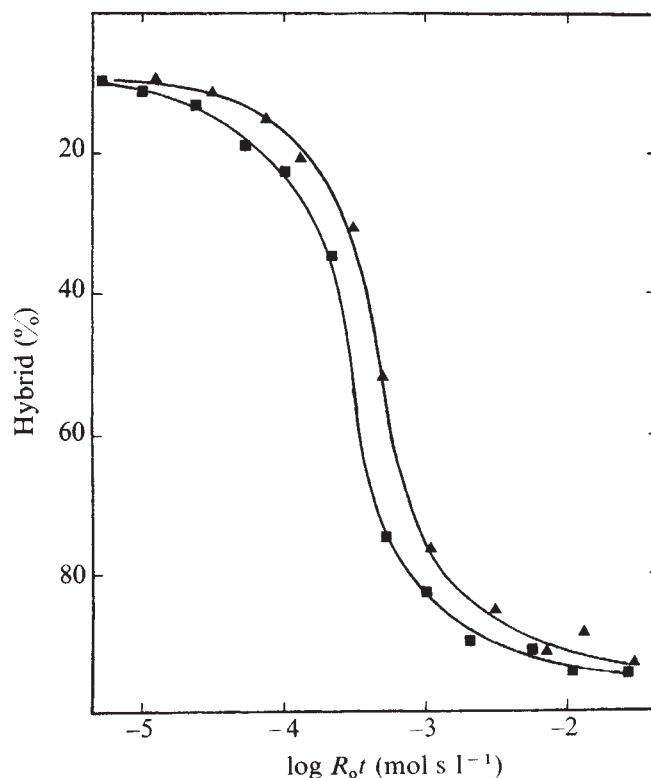
RNA added	R_0t (mol s l^{-1})	Hybridisation (%)
18S rRNA	6.8×10^{-1}	2.2
28S rRNA	7.2×10^{-1}	1.0
'Crude' globin mRNA	5.0×10^{-1}	85.4
'Purified' globin mRNA	5.5×10^{-1}	3.0
H5 mRNA	3.0×10^{-1}	89.0

H5 cDNA 2000 c.p.m. was hybridised to several electrophoretically pure RNA samples to the indicated R_0t values. The percentage of cDNA hybridised was then calculated by S1 nuclease assays. The percentage has been corrected by subtraction of the background due to S1-insensitive counts (about 4%). The 'crude' and 'purified' chicken globin mRNA refer to the same sample before and after purification by oligo(dT)-cellulose chromatography (see text).

preparations which have not been purified by oligo(dT)-cellulose chromatography⁴², contain a small amount of H5 mRNA. Because these hybridisations are done in a vast excess of RNA (compared with cDNA), sufficient of this contaminating H5 mRNA hybridises to protect 85% of the H5 cDNA from degradation by S1.

The kinetics and extent of the back-hybridisation of the H5 cDNA to excess, unlabelled H5 mRNA, can also provide some information about the fidelity of the copying of mRNA into cDNA. The kinetics of this hybridisation reaction are dependent on the sequence complexity of the mRNA and this can be estimated by comparison with a kinetic standard¹⁰. The standard used was the hybridisation of rabbit globin mRNA to its cDNA, and as determined by resistance of the hybrids to S1 nuclease, these hybridise in a sharp transition with a mid-point ($R_0t_{1/2}$) of

Fig. 4 Hybridisation of H5 mRNA and rabbit globin mRNA to their respective cDNAs. The reaction mixtures (50 μ l) contained 2,000 c.p.m. of H5 cDNA or 5,000 c.p.m. of globin cDNA, and varying amounts of mRNA. Hybridisation and S1 assays were as described¹⁰. ■—■, H5 mRNA-cDNA; ▲—▲, globin mRNA-cDNA.



5×10^{-4} (Fig. 4). This corresponds to a complexity of about 1,300 nucleotides for α plus β globin mRNA, since it is known that cDNA probes to these two mRNAs do not cross hybridise to any appreciable extent⁴³. In contrast, H5 mRNA hybridises to its cDNA with a similar sharp

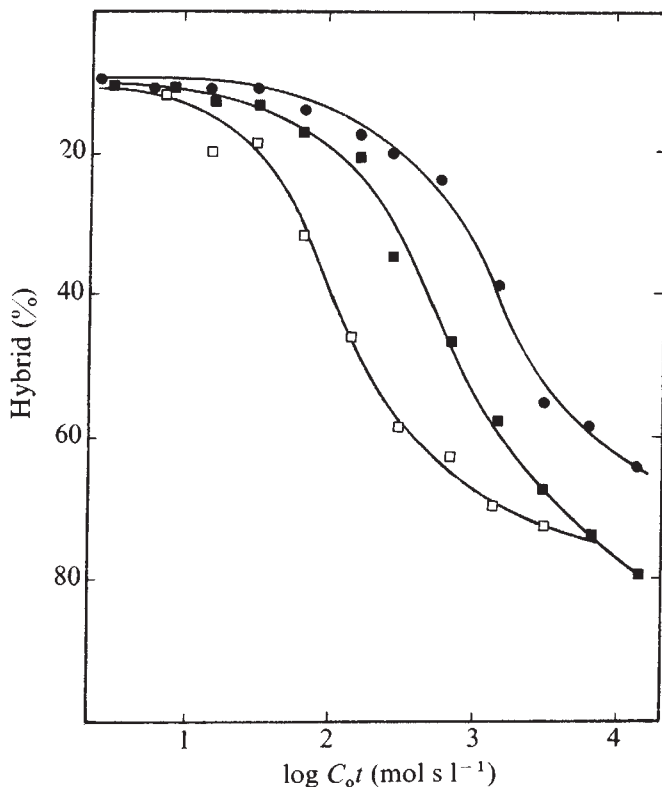


Fig. 5 Reassociation of H5 cDNA with excess chicken reticulocyte nuclear DNA. Three separate hybridisations were carried out in high salt buffer and the percent hybridisation assayed with nuclease S1 as described¹⁰. The three samples were: ●—●, ³H-chicken DNA-DNA; □—□, H5 cDNA-DNA; ■—■, chicken globin cDNA-DNA.

transition but with a $R_{0t_{1/2}}$ of about 2.8×10^{-4} (Fig. 4). This corresponds to a complexity of some 730 nucleotides which is the approximate size of the H5 mRNA (Fig. 1b). This indicates that H5 is coded by a single species of mRNA. The low $R_{0t_{1/2}}$ value and the sharpness of the transition also indicate that the H5 mRNA is not contaminated to any significant extent with other mRNAs.

H5 gene reiteration frequency

The reiteration frequency of the H5 gene can be estimated by annealing the cDNA from H5 mRNA with a vast excess of total chicken DNA³⁶. To determine the $C_{0t_{1/2}}$ for unique sequences, chicken globin cDNA was reannealed to chromosomal DNA and a mid-point ($C_{0t_{1/2}}$) of 1.2×10^3 was obtained (Fig. 5). This is very similar to the $C_{0t_{1/2}}$ for the reassociation of total chicken DNA unique sequences (Fig. 5 and ref. 6), and is indicative of the fact that there are only 1 or 2 copies of the globin genes in the chicken genome. The hybridisation of H5 cDNA to chromosomal RNA, on the other hand, has a $C_{0t_{1/2}}$ of 1.2×10^2 which means that the rate of the reaction is an order of magnitude faster than the unique rate. Since the globin and H5 cDNAs are of similar size and their G+C content is probably very similar (on the basis of the amino acid

analysis of the proteins), then this difference in rate must reflect a reiteration of the H5 gene in the chicken genome. The extent of reiteration is directly proportional to the $C_{0t_{1/2}}$ value compared with the kinetic standard³⁶, and so the H5 gene is reiterated about 10 times. Furthermore, as the hybridisation goes to over 70% completion, the minor contaminating species in the H5 cDNA are not responsible for the reaction.

We have therefore demonstrated that the gene coding for H5 histone is reiterated in the chicken genome as are histone genes in all other organisms examined. The extent of reiteration is similar to that found in man¹⁵ and mouse¹⁵ but far less than is found in sea urchins¹¹. The reason for the clustering of histone genes in sea urchins^{11,12,44} is unknown but may be involved in the coordinate control of histone synthesis or in the linkage of histone mRNA synthesis to DNA synthesis^{23,45,46}. Since H5 is subject to neither of these controls, and indeed is made only in red blood cells, the presence or absence of clustering of H5 genes with other histone genes may shed some light on this phenomenon. We are currently investigating the organisation of H5 histone genes in relation to other histone genes in the chicken genome.

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Organisation of the genes coding for 5S RNA in the Chinese hamster

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Hybridisation of iodinated mouse 5S RNA with Chinese hamster DNA fractionated on a CsCl gradient, identifies two separate DNA components that hybridise with the labelled probe. One component is slightly, and one considerably heavier than the main band DNA. This suggests that the gene coding for 5S RNA in the Chinese hamster may constitute two distinct populations differing in G+C content of their adjacent spacer regions.

THE organisation of the genes coding for ribosomal 5S RNA in eukaryotes has been best studied in the frog *Xenopus*^{1,2}. In this system the 5S genes occur as clusters of tandem repeats of alternating transcribed and non-transcribed nucleotide sequences which have been localised at the ends of most of the chromosomes by *in situ* hybridisation³. Analysis of the primary sequence of 5S ribosomal RNA (rRNA) has revealed two populations differing slightly in nucleotide sequence. One population is found predominantly in oocytes, the other in somatic cells^{4,5}. In contrast to *Xenopus*, information regarding the organisation of the 5S genes in mammals is sparse. The organisation of these genes in the Chinese hamster was studied by examining their buoyant density characteristics in isopycnic CsCl gradients and here I report the results.

DNA from Chinese hamster V79 tissue culture cells was centrifuged to equilibrium in neutral CsCl. The gradients were fractionated and the DNA in each fraction was denatured and trapped on nitrocellulose filters as described previously⁶. To localise the 5S genes within the gradients, the DNA in each fraction was hybridised with ¹²⁵I-labelled 5S RNA obtained from a mouse liver ribosomal pellet. The mouse 5S RNA was prepared by gel filtration on a 90-cm Sephadex G-100 column and on acrylamide gel electrophoresis ran as a sharp band, well separated from a tRNA marker. Iodination was by the procedure described by Prenskey *et al.*⁷, yielding an RNA with specific activity of $\sim 5 \times 10^6$ – 10^7 c.p.m. μg^{-1} .

Hybridisation with mouse 5S RNA

Mouse 5S RNA was selected as a probe for detecting the Chinese hamster 5S genes, so as to reduce the amount of hybridisation by RNAs contaminating the 5S RNA preparation. The nucleotide sequence of Chinese hamster 5S RNA (ref. 8) is virtually identical to that of other mammals examined^{9–12} so mouse 5S RNA should hybridise faithfully to the Chinese hamster DNA, whereas contaminating RNA degradation products which are copurified with the 5S RNA, should cross hybridise poorly, if at all.

When the fractionated Chinese hamster DNA was hybridised with the mouse ¹²⁵I-5S RNA preparation, hybridisation occurred as a single broad peak with a shoulder on the heavy side of the DNA (Fig. 1a). The position of the radioactivity is reminiscent of the position at which the Chinese hamster ribosomal genes band in neutral CsCl⁶, suggesting that in addition to hybridisation of 5S RNA sequences, hybridisation of rRNA degradation

products which had been copurified with the 5S RNA, and which should also effectively cross hybridise with Chinese hamster DNA was also being detected.

To test the extent to which labelled ribosomal degradation products were contributing to the hybridisation shown in Fig. 1a, the DNA from fractions of a parallel gradient, which had been prepared at the same time, was hybridised with the same number of input counts of labelled mouse 5S RNA, but this time in the presence of an excess amount of unlabelled Chinese hamster rRNA (25 $\mu\text{g ml}^{-1}$). The results (Fig. 1b) show that about half of the hybridised radioactivity was taken out

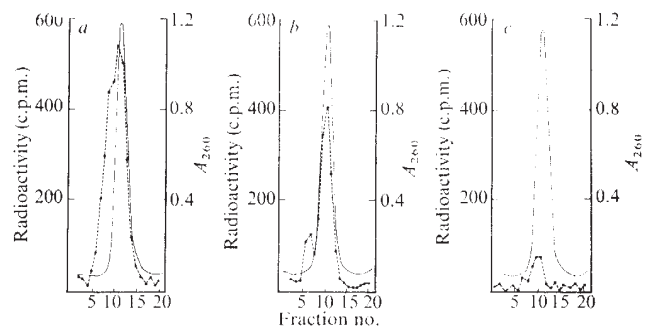


Fig 1 Hybridisation of iodinated mouse 5S RNA to Chinese hamster DNA. Chinese hamster DNA (50 μg) with a molecular weight of about 10^7 was fractionated in each of three identical CsCl gradients. The DNA of each fraction was denatured, trapped on nitrocellulose filters and hybridised with ¹²⁵I-mouse 5S RNA in the presence of the appropriate competing RNA. *a*, Chinese hamster DNA (50 μg) hybridised with a ¹²⁵I-mouse 5S RNA preparation (3×10^5 c.p.m. per 3 ml). *b*, Chinese hamster DNA (50 μg) hybridised with ¹²⁵I-mouse 5S RNA (3×10^5 c.p.m. per 3 ml) plus unlabelled Chinese hamster rRNA (25 $\mu\text{g ml}^{-1}$). *c*, Chinese hamster DNA (50 μg) hybridised with ¹²⁵I-mouse 5S RNA (3×10^5 c.p.m. per 3 ml) in the presence of unlabelled Chinese hamster rRNA (25 $\mu\text{g ml}^{-1}$) plus unlabelled *X. laevis* oocyte 5S RNA (20 $\mu\text{g ml}^{-1}$). —, A_{260} ; ●, radioactivity.

by competition, and that the radioactivity remaining resided in two discrete peaks, one considerably denser than the bulk of the DNA and the other only slightly heavier than the main band. To explore the possibility that these two peaks are due to hybridisation by sequences contained in 5S RNA, an additional competition hybridisation experiment was performed. The DNA in fractions from a third gradient, prepared at the same time as the previous two, was hybridised with the mouse ¹²⁵I-labelled 5S RNA preparation in the presence of unlabelled Chinese hamster rRNA (25 $\mu\text{g ml}^{-1}$) plus excess unlabelled 5S RNA (20 $\mu\text{g ml}^{-1}$) prepared from *Xenopus laevis* oocytes (a gift from Dr Ronald Brown). A 5S RNA competitor from such a distant evolutionary source was selected to reduce as much as possible the inclusion of RNAs that might contaminate the 5S RNA preparation and cross hybridise with the Chinese hamster DNA. If one of the radioactive peaks in Fig. 1b

represents hybridisation by a contaminating RNA in the mouse liver 5S RNA preparation, one would expect that in the presence of unlabelled rRNA and *Xenopus* oocyte 5S RNA one peak would disappear and the other, due to the contaminant, would remain intact. When the hybridisation reaction was performed in the presence of the two competing RNA populations, both peaks of radioactivity were virtually eliminated (Fig. 1b).

It is important to exclude any artefacts such as physical trapping of 5S DNA in the main band as the source of the hybridising material in the larger peak. To this end a series of four experiments, described in Fig. 2, was performed. Two 25-ml CsCl gradients each containing 1 mg of Chinese hamster DNA sheared to a molecular weight of about 5×10^6 daltons were fractionated, and the heavy regions of the gradients containing the smaller peak of hybridisation were retained. The heavy region of one gradient was mixed with 100 μ g *Escherichia coli* DNA and that of the second gradient was mixed with 100 μ g Chinese hamster DNA. The DNA mixtures were banded in neutral CsCl, the gradients fractionated, and the DNA in each fraction hybridised with 125 I-labelled mouse 5S RNA in the presence of a rRNA competitor. *E. coli* DNA has a 50% G+C content and as predicted bands at a density of 1.710 g cm^{-3} (Fig. 2a). The peak of 5S RNA hybridisation occurs at a density of 1.718 with no significant hybridisation associated with the bulk of the *E. coli* DNA, indicating that physical trapping of 5S DNA sequences in the gradient has not occurred.

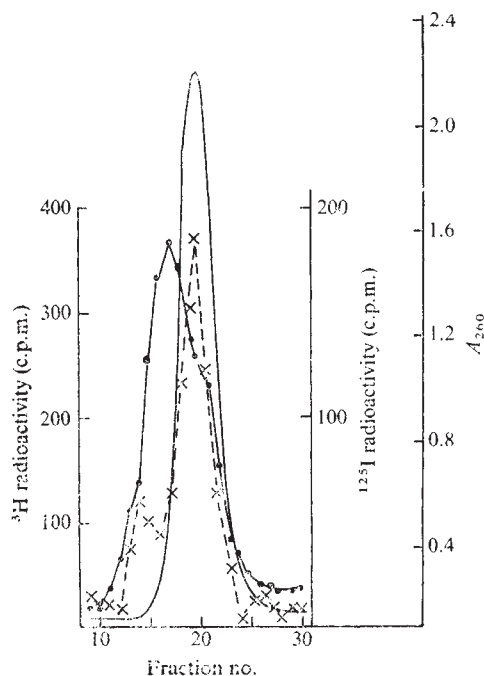


Fig. 2 Hybridisation to Chinese hamster ribosomal and 5S genes in a neutral CsCl gradient. A CsCl gradient containing 100 μ g Chinese hamster DNA was fractionated and DNA from each fraction was denatured and adsorbed on to nitrocellulose filters. The filters were cut in half and one set of filter halves was hybridised with 125 I-mouse 5S RNA in the presence of unlabelled Chinese hamster rRNA (25 μ g ml $^{-1}$). The second set of filter halves was hybridised with 3 H-ribosomal cRNA from *X. laevis*. —, A_{260} ; x — x, 125 I radioactivity; ● — ●, 3 H radioactivity.

source of the larger peak of radioactivity. When Chinese hamster DNA, containing the additional heavy DNA from the larger gradient, was banded in CsCl and hybridised, the bulk DNA banded as expected at a density of 1.700 (Fig. 2c). Hybridisation results in two peaks of radioactivity at 1.718 and 1.702, with the heavier peak substantially enriched. The important observation is that both radioactive peaks band faithfully at their predicted densities. In a situation where the region of a gradient containing the heavy peak has been removed and the DNA is rebanded in CsCl, only one peak of radioactivity at a density of 1.702 is revealed on subsequent fractionation and hybridisation (Fig. 2d). The combined results of these experiments confirm that the two peaks of radioactivity obtained on hybridisation with 125 I-labelled 5S RNA reflect true populations of DNA that band faithfully at densities of 1.718 and 1.702 respectively.

The possibility remains that one of the two radioactive peaks is due to residual hybridisation of labelled rRNA degradation products and that the added *Xenopus* 5S RNA in Fig. 1c contains sufficient rRNA sequences to complete the competition. This interpretation was eliminated by an experiment in which the DNA from fractions of a gradient was trapped on nitrocellulose filters and the filters cut in half. One set of filter halves was hybridised with mouse 125 I-labelled 5S RNA in the presence of excess unlabelled rRNA and the other set of filter halves was hybridised with a ribosomal 3 H complementary RNA (cRNA) synthesised from purified *Xenopus* rDNA (provided by Dr Donald D. Brown). The *Xenopus* ribosomal cRNA hybridises specifically with sequences complementary to 18S and 28S RNA within the Chinese hamster genome⁶. The results described in Fig. 3 show that the ribosomal genes band directly between the two peaks of radioactivity generated by hybridisation of the mouse 125 I-5S RNA preparation, thus excluding hybridisation by rRNA sequences as a source for either of the peaks. These results are also consistent with the observation that Chinese hamster rDNA has a buoyant density of 1.708 as determined by analytical ultracentrifugation of a partially purified rDNA preparation (unpublished data).

Organisation of 5S RNA genes

The data obtained from these experiments allow three interpretations. The first is that a mouse liver polysomal pellet and *Xenopus laevis* oocytes contain an RNA in common that is neither 5S RNA, rRNA nor tRNA, and which hybridises effectively with Chinese hamster DNA. Although unlikely, this possibility has not, as yet, been completely excluded.

A second interpretation would argue that 5S genes occur as a series of clusters within blocks of high G+C non-5S DNA. The rationale is that two peaks of hybridisation can be generated by random breakage of DNA which would result in pure 5S DNA molecules from the internal region of the cluster and a small fraction of 5S DNA molecules containing varying amounts of the external high G+C non-5S DNA. Such a distribution seems unlikely for at least two reasons. First, one would predict that as the DNA is sheared to a smaller size the amount of radioactivity contributed by the smaller peak should decrease, yet the ratio of small peak to large peak remains relatively constant for DNAs between 5×10^6 and 2×10^7 daltons. When the DNA is further sheared to between 5×10^5 and 7×10^5 daltons, hybridisation occurs as a single broad band with a peak of radioactivity at about 1.715 g cm^{-3} (unpublished). These data, however, cannot distinguish between a single component comprising a broad band with a peak at 1.715 g cm^{-3} and two components of different buoyant density that cannot be resolved. Second, since the fragments containing the external regions should be heterogeneous with respect to their content of high G+C DNA, one would expect such fragments to band as a broad smear in the heavy part of the gradient. In fact, however, the smaller peak of radioactivity bands sharply,

When *E. coli* DNA alone was banded in CsCl and hybridised with 125 I-labelled 5S RNA, no radioactivity was retained on the filters (Fig. 2b), eliminating the exchange of radioactive iodine from the RNA probe to the DNA on the filters as the

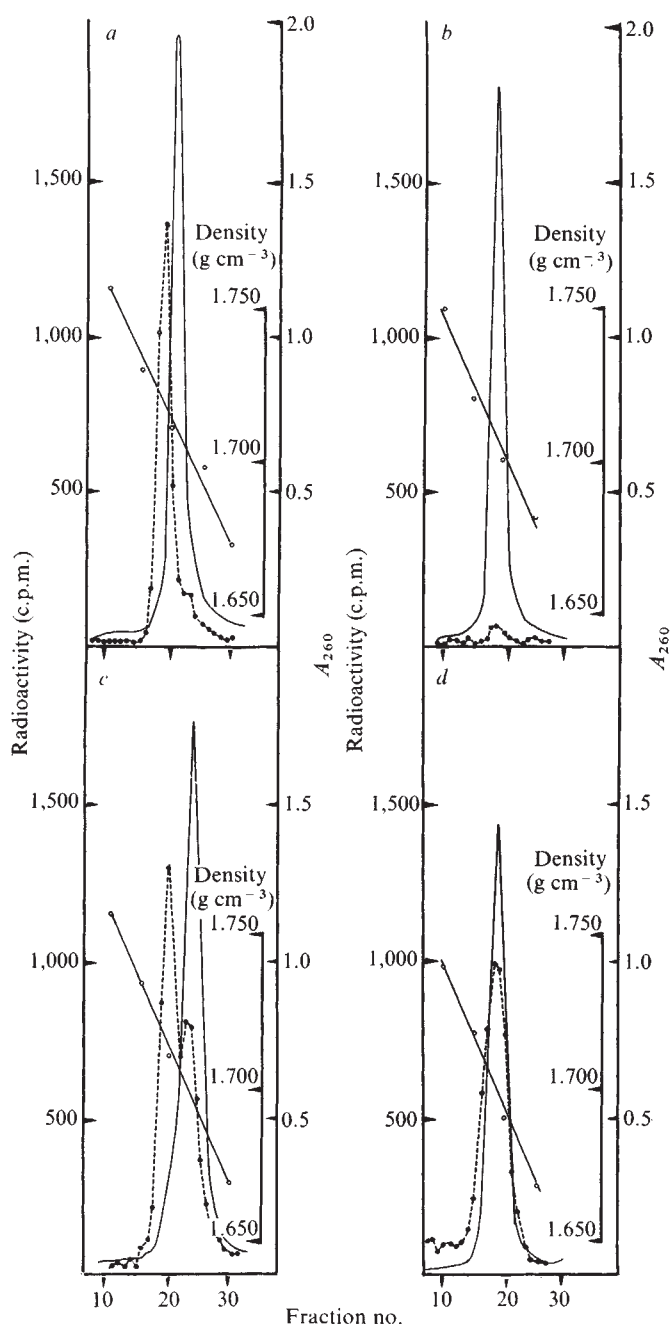


Fig. 3 Rebanded of the DNA hybridising with ^{125}I -5S RNA. *a*, Chinese hamster DNA banding at 1.718 g cm^{-3} from a 25-ml CsCl gradient containing 1 mg DNA (sheared to 5×10^6 daltons) was added to 100 μg *E. coli* DNA (about 10^7 daltons). This DNA mixture was recentrifuged in neutral CsCl, the gradient was fractionated, and the DNA was hybridised with mouse ^{125}I -5S RNA in the presence of rRNA competitor. *b*, *E. coli* DNA was banded in neutral CsCl, the gradient was fractionated, and the DNA in each fraction was hybridised with mouse ^{125}I -5S RNA in the presence of a rRNA competitor. *c*, Chinese hamster DNA banding at 1.718 g cm^{-3} from a 25-ml CsCl gradient containing 1 mg DNA (5×10^6 daltons) was added to 100 μg Chinese hamster DNA (5×10^6) and recentrifuged in CsCl. The gradient was fractionated, the DNA hybridised with mouse ^{125}I -5S RNA in the presence of a rRNA competitor. *d*, 100 μg Chinese hamster DNA was centrifuged in neutral CsCl and the region banding at 1.718 g cm^{-3} was removed. The remaining DNA was recentrifuged in CsCl, the gradient was fractionated and the DNA in each fraction hybridised with mouse ^{125}I -5S RNA in the presence of a rRNA competitor. All gradients were centrifuged at the same time in a Beckman 50 Ti rotor. The filters containing the denatured DNA from all four gradients were incubated together in a reaction mixture containing 50% formamide, $4 \times \text{SSC}$, Chinese hamster rRNA ($25\text{ }\mu\text{g ml}^{-1}$) and mouse ^{125}I -5S RNA ($1.2 \times 10^6\text{ c.p.m. per 12 ml}$) at 37°C for 30 h. The density profile of each gradient was determined from the refractive index of a 4- μl aliquot from every fifth fraction. — A_{260} ; — — —, radioactivity; O—O, density.

multiple populations of 5S genes since such heterogeneity has already been demonstrated in *X. laevis*^{4,5}, and since in human cells, 5S genes may be scattered in a large number of chromosomes¹³. In HeLa cells, molecular hybridisation studies with fractionated chromosomes have suggested that the 5S genes numbering about 2,000 per haploid genome¹⁴ are contained on chromosomes of all size classes with some enrichment in the largest size group¹³. *In situ* hybridisation with chromosomes of human diploid cells has suggested that a cluster of 5S genes is located on chromosome 1, although scattered grains were also observable over many of the other chromosomes^{15,16}. In *X. laevis*, two gene populations were detected by differences in the primary sequence of the 5S RNAs themselves^{4,5} rather than by differences in their spacer DNAs. Genes coding for 5S RNA have been isolated from both *X. laevis* (1) and *X. mulleri* (2) and in both cases, the spacer regions constitute most of the 5S DNA. Since the transcribed regions of *X. laevis* and *X. mulleri* 5S DNA comprise only 6 and 14% respectively of the 5S DNA², the behaviour of this DNA in isopycnic gradients depends more on the base composition of the spacer than the transcribed region. If the same relationship between spacer and transcribed DNA exists in the 5S DNA of mammals, subpopulations of this gene differing primarily in the base composition of the spacer should be detectable in isopycnic gradients and should yield similar results to those presented in these experiments.

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indicating a relatively homogeneous population of molecules.

A third interpretation of the results is that the Chinese hamster genome contains two populations of genes that code for 5S RNA but which differ in the G+C content of their adjacent spacer regions. Such differences in base composition of the spacer would confer different buoyant densities on the two gene populations in CsCl and would result in the two peaks of radioactivity generated by hybridisation. Since the G+C content of mammalian 5S RNA is 60% (refs 9–12), the corresponding DNA should have a buoyant density of 1.720 g cm^{-3} . The lighter population bands at 1.702 g cm^{-3} and should therefore have an A+T-rich spacer. The smaller and heavier population bands at 1.718 g cm^{-3} and would therefore be predicted to contain a spacer with a base composition much like that of the transcribed region. Unequivocal identification of both peaks of radioactivity as 5S genes will require the demonstration that the RNAs hybridising in each peak are 5S RNA, and experiments to that end are currently in progress.

It is not unreasonable to expect mammalian cells to contain

letters to nature

Origin of the black hole in Cyg X-1

THE evidence that Cygnus X-1 is a black hole of mass $\gtrsim 9M_{\odot}$ (ref. 1) is not conclusive, but is sufficient to warrant some consideration of the implications of such an object for the late stages of stellar evolution. We argue here that such a black hole must form directly by implosion of a star of mass $\gtrsim 30M_{\odot}$ rather than by accretion on to a neutron star. The Galaxy may contain $\sim 10^7$ such massive black holes.

Supernova models based on either the degenerate cores of intermediate mass stars ($4-8M_{\odot}$, see ref. 2) or the iron cores of more massive stars³, suggest a collapsing core of initial mass $\sim 1.4M_{\odot}$ and hence a final gravitational mass of $\sim 1.2M_{\odot}$. In the more massive stars the collapse of some material of lower atomic weight than iron might increase the final mass somewhat (W. D. Arnett, private communication). The estimated masses for the X-ray sources Her X-1 ($\sim 1.3M_{\odot}$, A. B. Middleditch and C. D. Nelson, unpublished), Cen X-3 ($0.6-1.1M_{\odot}$, ref. 1), and Vela X-1 ($1.6-2.1M_{\odot}$, refs 4, 5), which are almost certainly neutron stars because of their X-ray pulsations, accord well with these estimates and with recent calculations of the maximum mass of neutron stars $M_{\text{lim}} \sim 1.5M_{\odot}$ (ref. 6) to $2.4M_{\odot}$ (ref. 7). For sources without periodic X-ray variations, the mass estimates are less certain; however, with the exception of Cyg X-1, the estimated masses cluster $\sim 1-2M_{\odot}$ (see Table 1). In summary, all known binary X-ray sources other than Cyg X-1 have masses consistent with those expected for neutron stars somewhat below M_{lim} or black holes that are only slightly above M_{lim} .

One might envisage two alternative origins for a hole $\gtrsim 9M_{\odot}$ in Cyg X-1: either it was formed at essentially its present mass by the implosion of a massive star, or it began as a neutron star that grew to its present mass through accretion, during which growth it collapsed into a black hole when it passed M_{lim} . We first discuss the second possibility.

The time scale for growth by accretion is restricted by the Eddington limit. For

$$L_X \leq L_{\text{Edd}} = 4\pi c G m_H M / \sigma_T,$$

where σ_T is the Thomson cross section, we have

$$\tau_{\text{grow}} \equiv M/\dot{M} \geq \frac{c\sigma_T}{4\pi G m_H} f = 1.1 \times 10^8 f_* \text{ yr} \quad (1)$$

Here $f \equiv L/\dot{M}c^2$ and $f_* \equiv f/0.25$. Since many X-ray sources seem to radiate at nearly the Eddington limit, it is reasonable to assume that τ_{grow} will be near the lower limit given by equation (1). For black holes, we take $f_* = 1$ because expected efficiencies are $f = 0.42$ and 0.06 for maximally rotating and

non-rotating holes, respectively¹⁰. Therefore $\tau_{\text{BH}} \gtrsim 1.1 \times 10^8 \text{ yr}$. Since the companion of Cyg X-1 (HDE 226868) has a main sequence lifetime $\lesssim 5 \times 10^6 \text{ yr}$ for its inferred mass $\gtrsim 25M_{\odot}$ (ref. 1), Cyg X-1 cannot have grown from $M_{\text{lim}} \sim 2M_{\odot}$ to $\gtrsim 9M_{\odot}$ unless $f_* \lesssim 0.05$. There is no basis for assuming such a low efficiency, and we conclude that the black hole in Cyg X-1 formed, as such, at nearly its present mass.

Our conclusion, that the black hole of $\sim 10M_{\odot}$ in Cyg X-1 was formed directly in a stellar implosion, is somewhat surprising because recent work on stellar evolution has indicated that even very massive stars may form only neutron stars of $M \sim 1.2M_{\odot}$. Studies by Arnett³ suggest that massive stars end their lives with the collapse of an iron core of $M \sim 1.4M_{\odot}$, regardless of the total mass of the star. Most of these stars must explode leaving only a neutron star remnant, according to arguments involving the abundances of carbon and oxygen in the Universe.

Stars with $M \gtrsim 10M_{\odot}$ contain layers of carbon and oxygen which can potentially be ejected. By contrast, stellar evolution implies that intermediate and low mass stars can eject no carbon and oxygen; they either trap these elements in white dwarf remnants or convert them to iron peak elements in the process of core collapse and/or explosion. To provide enough carbon and oxygen to the interstellar gas, most stars with $M \gtrsim 10M_{\odot}$ must eject their CO layers rather than suffer a total collapse. Since the iron cores are nearly identical and their evolution and dynamics seem to be little affected by the total stellar mass, a tempting generalisation is to assume that all massive stars explode with the formation of a neutron star from the iron core.

The creation of a black hole in Cyg X-1 with an initial mass $\gtrsim 9M_{\odot}$ indicates that this generalisation is not correct. In spite of the homogeneity of Arnett's pre-collapse configurations, the direct formation of a black hole of $\sim 10M_{\odot}$ implies that an unknown factor, presumably associated with the increasing mass of the post-helium burning core, becomes important for sufficiently large stellar masses. This factor evidently alters either the pre-collapse configuration or the dynamics of the explosion.

A mass of $10M_{\odot}$ for the black hole is consistent with this picture. One expects the hydrogen envelope to have been transferred from the star which made the black hole to HDE226868 in the course of evolution, so that the object which finally collapsed was a bare core. A core of $\sim 10M_{\odot}$ would correspond to an initial main sequence mass of $\sim 30M_{\odot}$ (ref. 3) for the star which formed the black hole. The assumption that HDE226868 now has $M = 30M_{\odot}$ implies that it originally had a mass of $\sim 10M_{\odot}$.

According to Ostriker *et al.*¹¹, the local density of stars $\gtrsim 30M_{\odot}$ is $\sim 2 \text{ kpc}^{-2}$. If all such stars make black holes directly and one in ten occurs in a close binary, the density of such systems is consistent with the distance $\sim 2 \text{ kpc}$ to Cyg X-1 (ref. 12). Many systems would spend their entire main sequence lives obscured within the dense clouds that gave them birth. Over its lifetime, the Galaxy would have produced $\sim 10^7$ massive black holes.

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Table 1 Estimated masses of binary X-ray sources

Name	Mass $M/M_{\odot}$	Ref.
3U1956+35 Cyg X-1	≥ 9	1
1118-60 Cen X-3	0.6-1.1	1
1653+35 Her X-1	1.3	*
0900-40 Vela X-1	1.6-2.1	5
1700-37	1.3 ± 0.2	8
0115-37 SMC X-1	~ 2	8
1617-15 Sco X-1	< 2	9
2030+40 Cyg X-3	$\sim 1(?)$	12
2142+38 Cyg X-2	$\sim 1(?)$	12

*A. B. Middleton and C. D. Nelson, unpublished.

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Production of heavy elements in nature

We shall describe here a possible mechanism for the production of heavy elements (with $A > 60$): these elements, we suggest, may be formed in the matter ejected from the envelope of a neutron star during the starquake process.

The r -process (rapid neutron capture) is known to be capable of forming heavy elements, and is believed to take place during a supernova outburst¹. Calculations^{2,3} of a supernova outburst show, however, that the neutron fluxes might be too low to explain the synthesis of heavy elements. Because of this, neutron stars are, in our opinion, among the most promising sites for the nucleosynthesis of heavy elements.

The mechanism of heavy element formation by the fission of the even heavier elements which can exist in neutron-rich nuclear fluid, was proposed by Goeppert-Mayer and Teller⁴ in 1947. The discovery of pulsars and their interpretation as neutron stars has provided the ideal initial conditions for the Mayer-Teller model^{5,6}. The existence of heavy neutron-rich nuclei was discussed by Zel'dovich⁷ and by Brueckner⁸, who considered neutron droplets in conditions of high pressure. Afterwards the chemical composition of neutron-star envelopes at densities $\rho = 10^{10}$ – 10^{12} g cm⁻³ was considered^{6,9,10}. In such conditions they are made of nuclei with $A \sim 200$ – 500 and free neutrons.

We consider the case where instabilities cause the ejection of matter from the neutron star surface. It will be shown that the evolution of ejected matter ends with the formation of heavy (even superheavy) elements¹¹. By contrast with ref. 4, matter composed of neutron-rich nuclei rather than of neutrons only will be considered. The heavy nuclei obtained in this way will be ejected from the magnetosphere of a neutron star, and may enrich interstellar space¹².

Initially we have nuclei with (Z, A) , as shown in Fig. 1, point a . The chemical potential of electrons decreases as the matter expands,

$$\mu_e \sim mc^2 \left(\frac{\rho}{\mu_o \times 10^6} \right)^{1/3}$$

where μ_o is the molecular weight of electrons, and is given by

$$\mu_o = \frac{\sum n_i A_i}{\sum n_i Z_i}$$

The (Z, A) nuclei decay by β emission and the new nuclei $(Z+1, A)$ will capture free neutrons. We assume that on the boundary of stability, the relation $Z/A = 1/4$ holds.

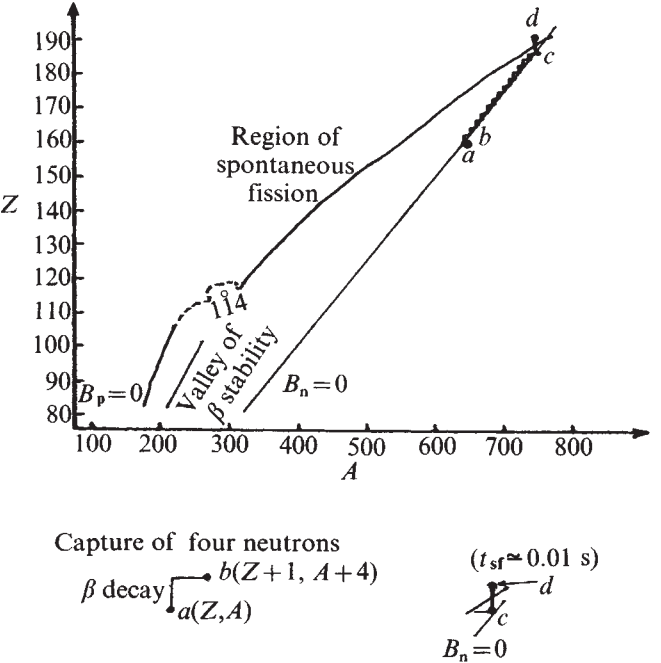


Fig. 1 Stability of superheavy nuclei. A nucleus at point a moves by β decay, and capture of neutrons (see inset) to point d , where it undergoes fission.

For the original nuclei at $\rho = 10^{10}$ g cm⁻³ we have $Z = 161$, $A = 644$ according to ref. 6. So, the β decay of the (Z, A) nuclei will be followed by four neutron captures by nuclei $(Z+1, A)$ —point b of Fig. 1. The following conditions on the characteristic times will be satisfied: $t_h \leq t_\beta$, $t_\beta > t_n$, where t_h , t_β , and t_n are the time scales of hydrodynamic expansion, β decay and (n, γ) reactions, respectively. These time scales are defined by: $t_h = 446a/\sqrt{\rho}$ in seconds, where a is an arbitrary scaling parameter,

$$t_n = \frac{m_H}{\alpha \rho \langle \sigma v \rangle}$$

where $m_H = 1.67 \times 10^{-24}$ g, α is the partial weight of neutrons in the matter,

$$t_\beta = \frac{6\Delta}{10^{-4} W_o^6}$$

where Δ is the density of nuclear states (in all cases we assume $\Delta = 15$) and W_o is the nuclear mass difference. We use here the notation introduced in ref. 1. For matter in these conditions, the neutron capture cross section $\sigma \sim 10^{-28}$ cm², mean velocity of neutrons $v \sim 10^8$ cm s⁻¹, so

$$t_n = \frac{10^{-7}}{\alpha \rho}$$

The calculated t_β values are given in Table 1. The characteristic time t_β does not depend on density, and t_n is inversely proportional to density. Nuclei moving along their 'evolutionary'

Table 1 Half lives for β decay of extremely heavy elements in seconds													
Z	160	161	162	163	164	165	166	167	168	169	170	171	172
A	640	644	648	652	656	660	664	668	672	676	680	684	688
t_β	0.011	0.011	0.011	0.012	0.012	0.012	0.013	0.013	0.014	0.014	0.015	0.015	0.016
Z	173	174	175	176	177	178	179	180	181	182	183	184	185
A	692	696	700	704	708	712	716	720	724	728	732	736	740
t_β	0.016	0.017	0.018	0.018	0.019	0.020	0.020	0.021	0.022	0.023	0.024	0.025	0.025

Table 2 Logarithms of spontaneous fission half lives (for $Z = 184$, $t_{sf} = 0.014$ s)

Z	160	161	162	163	164	—	181	182	183	184	185	186
A	640	644	648	652	656	—	724	728	732	736	740	744
$\lg(t_{sf})$	18.073	19.899	13.697	18.486	12.284	—	5.7685	-0.433	-4.3555	-1.8460	2.9425	-3.2590

path increase their atomic number Z and approach the region of spontaneous-fission instability. The spontaneous-fission half lives (t_{sf}) were calculated using the formula¹³

$$t_{sf} = 10^{-21} \times 10^{7.85 E_b(s)}$$

where

$$E_b = 19.0 - 0.36Z^2/A + \varepsilon \text{ (MeV)}$$

is the fission barrier, $\varepsilon = 0.7$ for odd-odd nuclei, $\varepsilon = 0.4$ for odd- A nuclei, and $\varepsilon = 0$ for even-even nuclei. Calculated half lives are given in Table 2. When nuclei reach the fission region (point d on Fig. 1) multiple fission might occur (triple or even more). Fission fragments will capture neutrons and some r -process-like recycling will take place.

Calculations on this model lead to the following picture. Within 0.4 s after the ejection of matter (at t_0) from the star, more than half the original nuclei with $Z = 161$, $A = 644$ move on their evolutionary tracks up to the point with $Z = 184$, $A = 736$, where spontaneous fission occurs. Half a second after the initial ejection, almost all the nuclei have done this. The density of matter at $t = t_0 + 0.4$ s and $t = t_0 + 0.5$ s is 5×10^6 and 3×10^6 g cm⁻³, respectively, and the characteristic times t_n are 2×10^{-14} and 3×10^{-14} s, respectively, so that $t_n < t_\beta$.

Both the fission fragments and the products of β decay can be in excited states. A probable means of de-excitation of these nuclei may be the evaporation of some neutrons, leading to the formation of neutron-deficient nuclei. The probability for such process is low but the abundance of neutron-deficient nuclei amounts to 10^{-4} – 10^{-5} of that of heavy nuclei¹⁴.

The described picture is in a good agreement with an estimate of the number of neutron stars in the Galaxy. To obtain the observed density of heavy elements, we need 10^9 neutron stars assuming that 10% of their envelopes form heavy elements and are ejected from the stars. In this way the neutron star may enrich with heavy elements the surfaces of stars in its neighbourhood. This mechanism may explain the presence of promethium on the surface of some stars. The longest lived isotope of promethium is ¹⁴⁵Pm with $t_{1/2} = 17.7$ yr. Lines of Pm have been found^{15,16} in the spectra of some Ap stars, but confirmation is needed. Another prediction of this model is the correlation between the γ -ray (of energy 2 keV–10 MeV) and heavy elements outburst.

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Lunar effect in the quiet-time D_{st} index

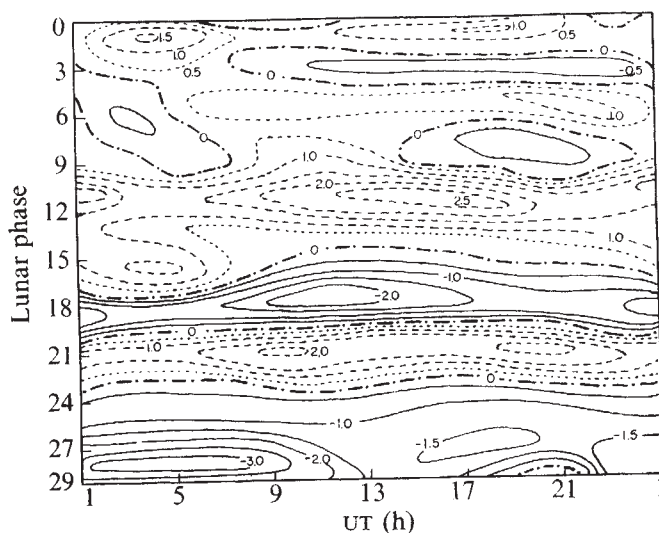
POLARISATION electric fields produced in the dynamo region should, theoretically, have a considerable effect on the distribution and energy of magnetospheric particles, but none has been found previously^{1–3}. Here we show that the D_{st} index calculated by Sugiura and Poros⁴ shows a semidiurnal variation, which we attribute to the effect of tides (caused by the Moon) in the dynamo region, thus verifying the theory.

The data used are the hourly values of the D_{st} index on five international quiet days in each month for 14 years (1959–72), the total number of data points being 21,000 ($25 \times 5 \times 12 \times 14$). These values are obtained for each hour of universal time (UT), and are dependent on the month of the year and also on the lunar phase. We have checked carefully that the data were collected uniformly over the lunar phases.

We have found a semiannual variation in the D_{st} index which will be discussed elsewhere. Our discussion is here limited to the relation of the D_{st} index to UT and to the lunar phase, so that we have used the data averaged over the whole month and whole year.

Figure 1 shows the distribution of D_{st} values with respect to UT and lunar phase. It is found that the D_{st} values show a systematic change with lunar phase, such that the positive and negative values appear alternately. To see more, the D_{st} values are rearranged in 'geomagnetic lunar time', that is, in hours measured from the lower transit of the Moon across the meridian containing the north magnetic pole. This is shown in Fig. 2, where the abscissa shows the lunar time relating to the geomagnetic axis: at 0 h the geomag-

Fig. 1 Distribution of the D_{st} index with respect to the lunar phase and UT (in units of nT).



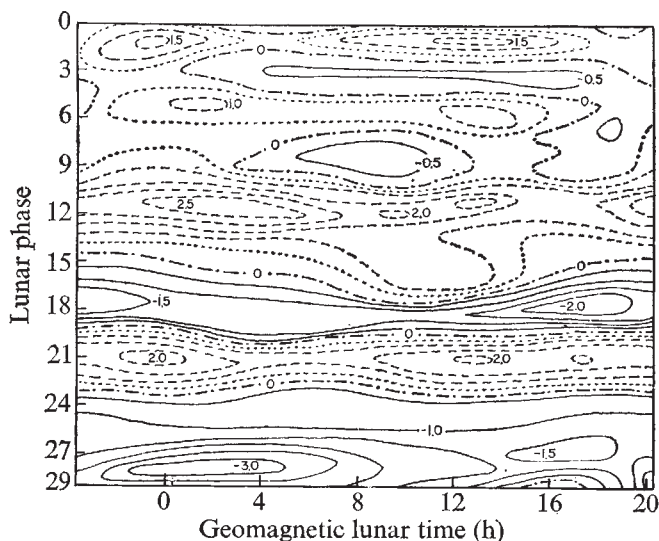


Fig. 2 Distribution of the D_{st} index with respect to the lunar phase and geomagnetic lunar time (see text for details). The figure is derived from Fig. 1 by shifting the values at each lunar phase to the left.

netic north pole is located opposite to the Moon with respect to the geographic pole. It may be seen that the D_{st} index changes systematically with lunar phase and lunar time. On averaging the values for geomagnetic lunar time over the lunar phases, we get Fig. 3, which clearly shows a semi-diurnal variation of the D_{st} index. The amplitude is ~ 0.3 nT, and the phase is such that the intensity of the westward ring current is minimum at 0 h and 12 h, and maximum at 6 h and 18 h; that is, the ring current is enhanced most when the Moon is at right angles to the meridian containing the geomagnetic pole. We have applied this method of analysis to the data of different periods (1958–69, 1959–70, 1960–71, 1961–72) and found results very similar to the above.

Although the D_{st} index derived from geomagnetic variations on the ground may include various effects, the main contribution seems to come from the ring current. We

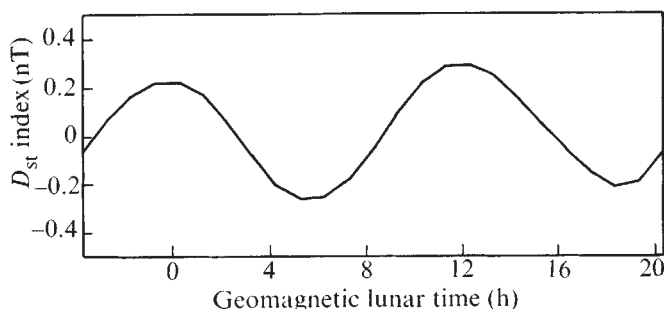


Fig. 3 Variation of the D_{st} index with geomagnetic lunar time.

cannot yet confirm whether the results obtained here can be explained by the electric field communicated from the dynamo region, but the Moon may have a significant effect on the ring current in the magnetosphere, or on the electric current in the ionosphere or Earth's core. A fuller account will be published elsewhere.

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Evolution of periodicities exhibited by fluctuations in the intensity of chorus

FROM visual analyses of the dynamic (frequency–time) spectra and recordings of the integrated intensity of chorus, a very-low frequency electromagnetic radiation which occurs naturally in the Earth's magnetosphere, it has been ascertained^{1–3} that chorus exhibits periodic or quasi-periodic characteristics arising from (1) periodic spacing of individual or groups of emissions (rising tones) or (2) amplitude modulation of the emissions or (3) both. These investigations have, however, been more qualitative than quantitative.

Here, the power spectral analysis method^{4,5} is used to investigate, quantitatively, the evolution of the periodicities exhibited by the intensity fluctuations of a 60-min recording of chorus. The recording was made at St John's Stadan Station, Newfoundland, Canada ($L=3.5$) on March 7, 1970, at 0850 UT. The recording was started close to the peak of an intense chorus event which lasted for more than 2 h. The short (2–3 s) period fluctuations were found to be attributable to the 2-hop travel time of Whistler mode echoes of the chorus emissions, whereas long (8–16 s) periods were attributed to amplitude modulation by geomagnetic micropulsations.

The dynamic spectrum of the chorus was first obtained using a Rayspan spectrum analyser. A visual examination of the dynamic spectrum revealed that intense emissions of the chorus were more closely spaced in the earlier parts of the recording than in the later parts, indicating comparatively more rapid amplitude modulation during the earlier part of the chorus recording. There was, however, no significant change in the major frequency band (~ 1.5 –3 kHz) occupied by the chorus emissions throughout the recording. Figure 1 shows the dynamic spectra of samples of earlier and later parts of the chorus recording.

Next, the chorus was filtered in the narrow frequency band (2–3 kHz), and the filtered output integrated using a time constant of 0.25 s. The integrated waveform was smoothed by means of a d.c. bandpass filter which attenuated statistical fluctuations with periods <1 s, and removed any ultra-flow frequency trends with periods >100 s, which are inimical to power spectral analysis, in the integrated waveform. The smoothed waveform was sampled at a rate of 4 s^{-1} and digitised to obtain a time series showing the temporal fluctuations in intensity of the chorus.

The chorus time series was divided into sections of 2,000 points (corresponding to 500 s); normalised power spectra were then computed, sequentially, for the sections with a running overlap of 1,000 points (250 s) to investigate any evolution of the dominant modes present in the 60-min piece of chorus. Figure 2a–c shows the twelve sequential power spectra computed for the data. There is a 9-min break between spectra 4 and 5. The break represents a section of the recording which contained interference.

Figure 3 is a contour map showing, effectively, the evolution of the twelve power spectra computed. The contours were obtained by the following procedure: First the normalised values of the spectral points, especially those defining peaks, in each of the sequential power spectra were denormalised by simply multiplying them by the variance (mean square value) of the corresponding time series used to compute the spectrum. The resultant values were renormalised with respect to the largest value (corresponding to the power in the most dominant period in the entire 60-min recording of chorus) in the set of twelve power spectra, and the values scaled to lie between 0 and

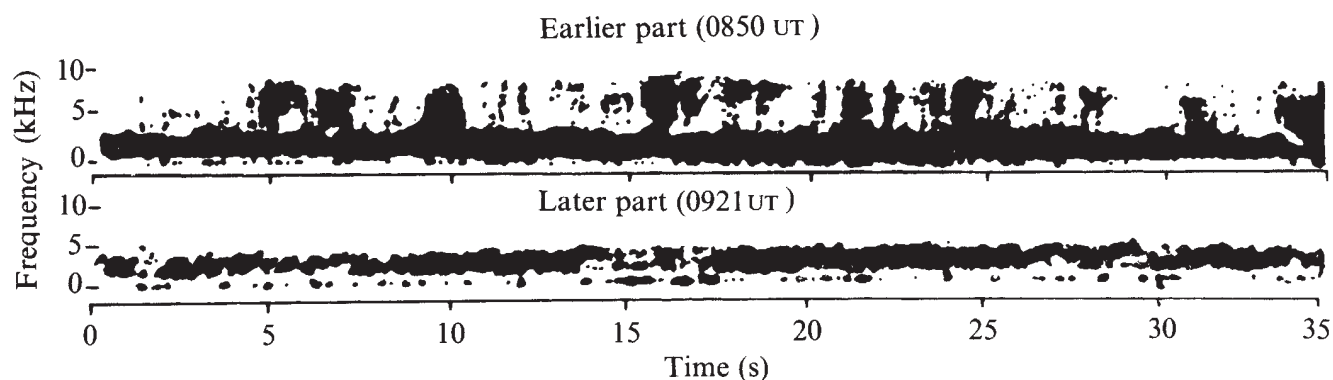


Fig. 1 Dynamic (frequency-time) spectra of samples of earlier and later parts of chorus.

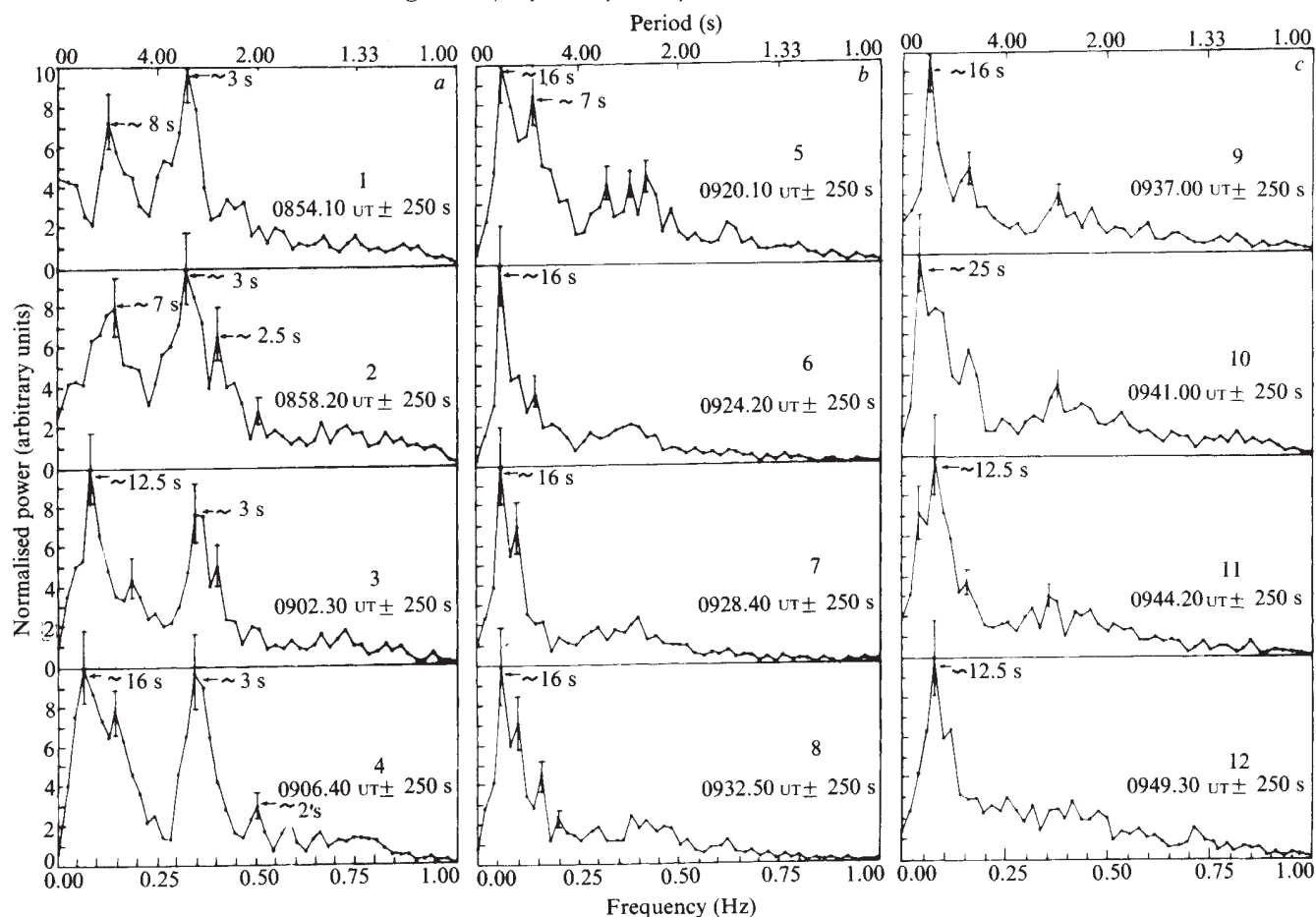
10. These scaled values were plotted for each power spectrum as shown by the dots and crosses in Fig. 3, and contours drawn by eye. Significant power peaks or ridges are identified on the contour plot as being high, H; significant valleys are identified as being low, L.

It is clear that there is a transition from more complex power spectra for earlier parts of the chorus to less complex ones for later parts. The transition time is ~ 25 min. In the more complex spectra, large spectral peaks defining both short (2–3 s) and comparatively long (8–16 s) periods are present whereas in the less complex spectra, the short period peaks are absent or have small amplitude suggesting that long periods tend to dominate the later parts of chorus.

From a study of chorus recordings from South Uist, Outer Hebrides, Scotland (S.K.A., unpublished), it was

found that short period (< 4 s) fluctuations in the intensity of chorus could be attributed to the 2-hop travel time of whistler-mode echoes of individual (or groups of) chorus emissions whereas comparatively long period (> 4 s) fluctuations are attributable to modulation by geomagnetic micropulsations of the same period. The dominant short period of 3 s observed here is equal to the typical 2-hop travel time of a 2-kHz (the mid-frequency of the chorus examined here) whistler mode echo propagating along the $L=3.5$ field line, within the plasmopause. The presence of short period fluctuations in intensity of the earlier parts of the chorus suggests that at the peak of the entire chorus event, the emissions have large energy and are able to echo (in the whistler mode) back and forth between conjugate hemispheres perhaps several times before dying off. It also suggests that at the peak of the chorus event some of the

Fig. 2 *a-c*, sequential power spectra of chorus time series.



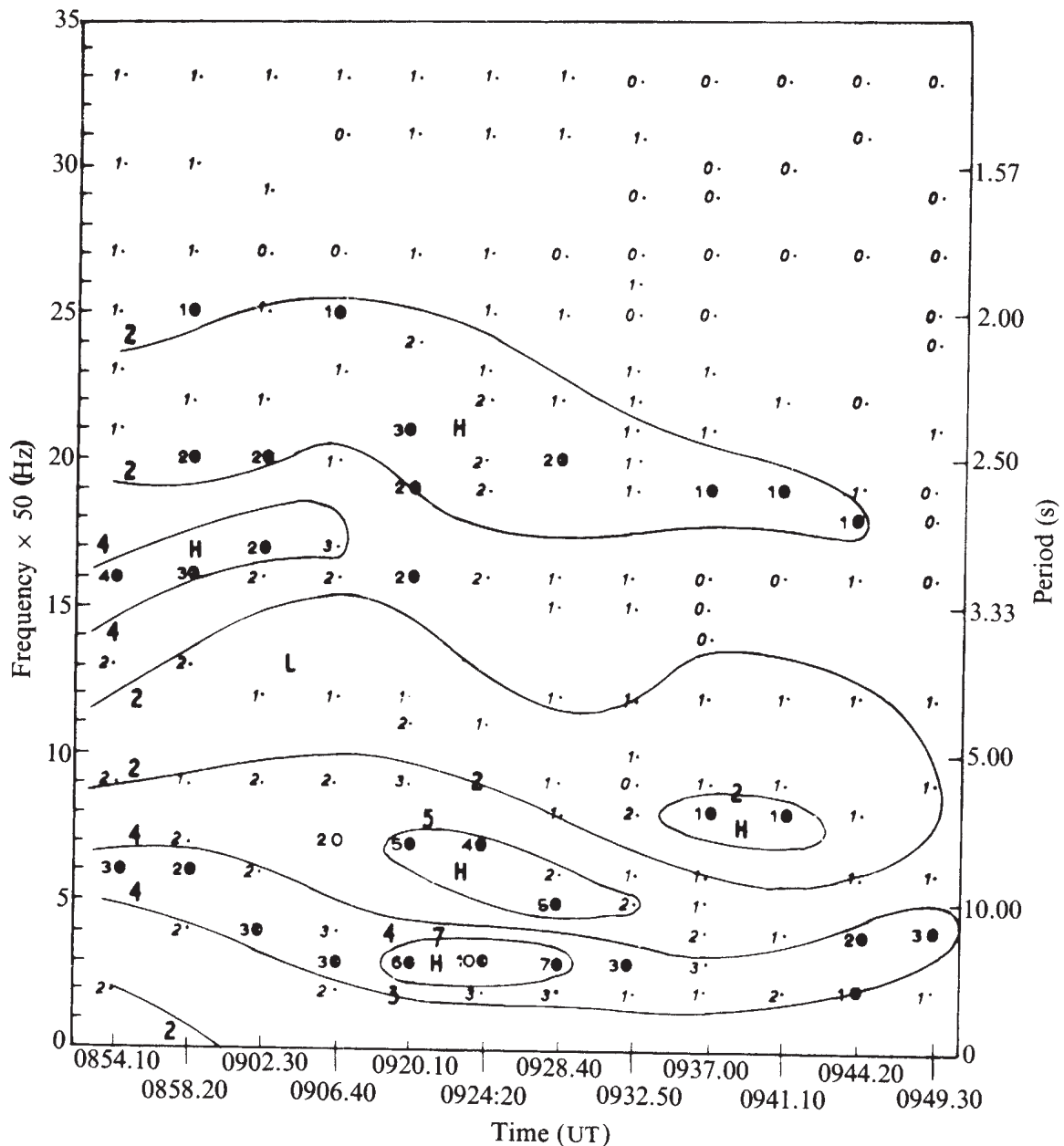


Fig. 3 Contours showing evolution of power spectra.

emissions were being generated inside the plasmopause.

Towards the end of the chorus event, the emissions will have little energy to echo, and so fluctuations in intensity of the chorus will be largely due to amplitude modulation by micropulsations (if present) of the magnetic field line along which the emissions (whistler mode waves) propagate. This kind of modulation, according to Coriniti and Kennel⁶ is exponential even for small amplitude whistler mode waves. The dominant long period fluctuation for later parts of the chorus is ~ 16 s; this is in fair agreement with the values of 14 s and 18 s for the eigenperiods of oscillation of the $L=3.5$ field lines, computed by Orr and Matthew⁷, assuming toroidal and poloidal oscillations respectively, for an electron density of 15 cm^{-3} . The low value used for the electron density is typical for a region outside the plasmopause. Thus, it would appear that near the end of the chorus event, the emissions were mostly being generated outside the plasmopause. The presence of short (2–3 s) and comparatively long (8–16 s) periods in power spectra for earlier parts of the chorus time series also supports generation of the emissions in a region close to the plasmopause.

In this case it is possible for the emissions to have been generated or alternatively travelled, some inside, and some outside, the plasmopause.

The evolution of the power spectra of the chorus time-series is, indeed, suggestive of changes in the energy of the plasma medium, available for conversion into chorus emissions. According to the Kennel–Petschek theory⁸, the energy of electrons resonant with whistler mode waves is dependent on the magnetic energy per particle, $E_e (= B^2/2\mu_0 N)$. Since the value of the magnetic field B is dependent on the L value of the field line, and the value of the electron density N at the point in the magnetosphere where gyroresonance between the waves and the electrons is taking place depends mainly on whether the point is inside or outside the plasmopause, the evolution observed here may possibly be related to the movement of the position of the plasmopause on a time scale of < 1 h.

The period of micropulsations of any particular geomagnetic field line depends on the plasma density and thus would be affected by changes of the plasmopause position. Thus, one would expect a similar evolution of the power

spectra of geomagnetic micropulsations. It is rather unfortunate that a simultaneous recording of geomagnetic micropulsations was not made at the time of the chorus event to enable a computation of the power spectra of the micropulsation time series to be made and thus to find out whether periods, and an evolution similar to those of the chorus time series would be exhibited. Nevertheless, a similar evolution of power spectra of the time series of micropulsations has been recorded elsewhere (S.K.A., unpublished).

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Large scale Palaeozoic shear zone in Australia and present extension to the Antarctic Ridge

THE Lower Proterozoic craton of central Australia is bounded to the south and south-east by shallow geosynclinal sediments of late Proterozoic to early Palaeozoic age. During the Palaeozoic these rocks were deformed into a series of upright folds, the trend of which follows the edge of the craton except in the area north and east of Adelaide where it swings to north-north-west-south-south-east, normal to the edge of the craton (Fig. 1). An analysis of this major structure gives an indication of a component of plate movement during the Palaeozoic.

The sigmoidal curve in the trend of the Palaeozoic folds has the form of a ductile shear zone analogous to the small scale, ductile shear structures described from deformed igneous rocks¹. This is confirmed by the parallelism of isogons drawn for the regional change in axial plane and cleavage orientation (Fig. 2). It suggests that a regional pattern of folds on north-east-south-west axial planes was modified by a sinistral shear couple parallel to the trend of the isogons (Fig. 2).

The simple shear mechanism is also confirmed by the variations in the intensities of deformation. Finite deformation increases as the fold axial planes change in orientation from north-east-south-west to north-north-west-south-south-east. Where the rocks have a north-east-south-west trend, the folding of the cover sequence is largely controlled by the position of structures in the underlying basement which tightened during Palaeozoic deformation. Thus, in the area west of Broken Hill, in the north-eastern sector of South Australia, anticlines in the cover sequence lie over much tighter earlier, antiforms in the basement gneisses. The intensity of deformation in the cover sequence varies locally; conglomerate pebbles at the base of the Upper Proterozoic sequence show the most intense deformation in synclinal keels, where the sediments are pinched into the basement gneisses. In the anticlinal areas, the pebbles show no apparent change of shape, and the sediments may show no cleavage. East of Adelaide, where the folds have a north-north-west-south-south-east trend, folding is more regular and better developed, conglomerate pebbles show

more intense deformation throughout the fold structures, and most of the rocks have a well developed cleavage. Deformation is heterogeneous, presumably as a result of the simple shear acting on an originally heterogeneous deformation pattern.

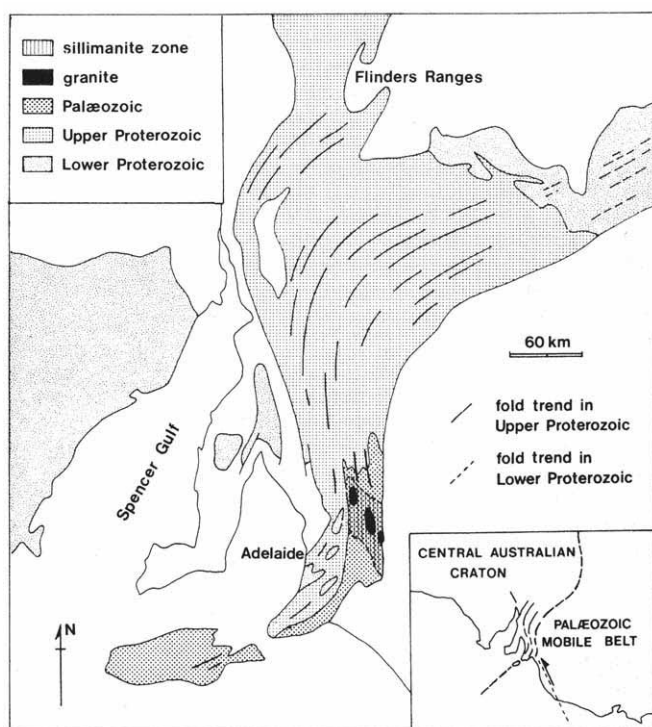
The intensity of deformation attributable to the simple shear component can be estimated from the change in orientation of the fold axial planes or cleavage, relative to the shear direction¹. Thus, the shear strain can be computed throughout the zone, with a maximum shear strain, γ , of 1.7 (a strain ratio of 4.5:1) some 30 km north-east of Adelaide (Fig. 2).

The zone of most intense shear strain coincides with a zone of sillimanite-grade metamorphism and migmatization (Fig. 1). The high heat flow required to give this local increase in metamorphic grade may result partly from intense shear strain; or alternatively, the position of the shear zone may have been controlled by the increase in ductility over a region of high heat flow. Evidence for that hypothesis has been given by Fleming and Offler²; they showed that the high grade metamorphism began before the development of the first cleavage, and continued through two later phases of localised deformation of crenulation cleavage.

The ductile shear zone is 150 km wide, and shows left-lateral displacement of the order of 150 km (Fig. 1). In terms of plate tectonics, the zone cuts across the edge of the Australian craton and must be intracontinental; the eastern part of the Australian plate must have moved northwards some 150 km relative to the western part. Seismic evidence indicates that there may be a gradual change in crustal structure under the northern extension of this shear zone³, and studies of electrical conductivity have detected an anomaly under the Flinders Ranges in the northern part of the zone. This may reflect a change in rock fabric at depth⁴.

The ductile deformation along the shear must have ceased during the Palaeozoic; it was presumably most active during the mid-Palaeozoic at the time of high grade

Fig. 1 Geological map of part of South Australia showing the sigmoidal trend of the fold axial planes and the area of high grade metamorphism.



metamorphism and granite production. Large faults, however with a similar north-west-south-east trend to the shear, postdate the deposition of Permian sediments.

From examinations deep crustal seismic profiles, Burwash *et al.*⁵ suggested that major faults and shear zones in the Churchill Province of Canada extend down to the mantle, and Beach⁶ claimed that the major shear in the Laxford region of north-western Scotland almost certainly penetrated to the mantle. According to Beach⁶, shear zones in north-western Scotland extend for a distance of the order of 10 times the amount of displacement. By analogy, the South Australian shear zone, with a displacement of 150 km can be expected to extend for some 1,500 km. Shear zones of such size most certainly affect the base of the lithosphere, and the change in rock fabric in the upper mantle⁴ beneath the Flinders Ranges presumably represents such deformed asthenosphere.

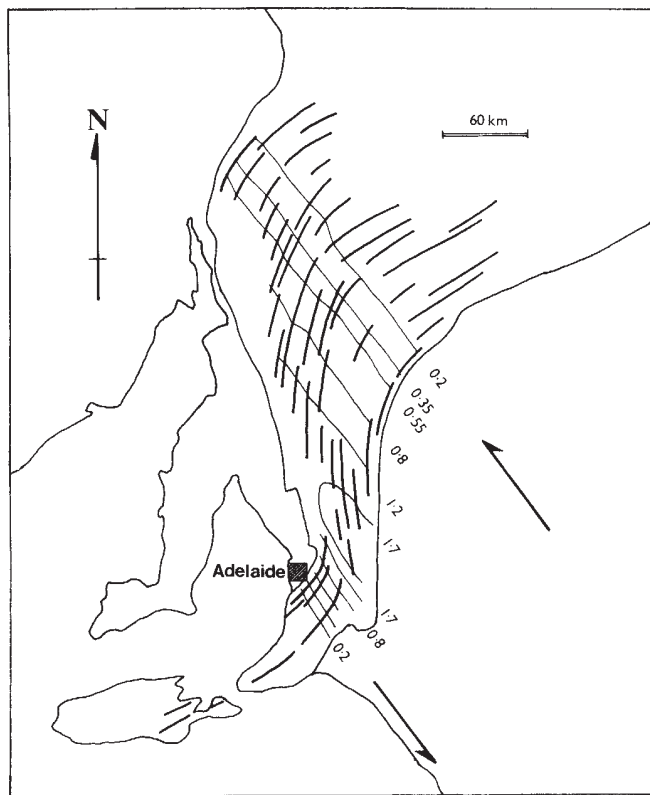


Fig. 2 Isogons drawn for the regional change in the orientation of fold axial planes. Estimated shear strains are shown.

Watterson⁷ has pointed out that the development of fabric and grain size reduction within shear zones produces a localised strain-softening effect so that major shear zones may thus be active persistently throughout geological time. Doyle *et al.*⁸ have shown that the Adelaide region of South Australia is a zone of recent seismic activity. Cleary and Simpson⁹ have extended this zone of seismicity to a seismic zone and a major transform fault on the Antarctic Ridge. They consider that the whole zone from South Australia to the Antarctic Ridge represents a zone of left-lateral differential shear movement formed by differences in spreading rates between different parts of the Australian plate. This is in accord with the suggestion of Wilson¹⁰ that transform faults on mid-oceanic ridges originate on lines of weakness within continents. Thus, the transform fault on the Antarctic Ridge was formed where the vertical zone of sheared lithosphere and asthenosphere was crossed

by a spreading ridge along which there was a gradient in the spreading rate.

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Magnetic inclination of basaltic lavas from the Mid-Atlantic Ridge near 37°N

DURING the course of the French-American Mid-Oceanic Undersea Study (FAMOUS), many samples of basaltic pillow lava were collected from the axial region of the Mid-Atlantic Ridge near 37°N. The vertical axes and the tops of nine of these samples have been determined fairly accurately, allowing calculations of magnetic inclination at the time of the cooling of the lavas to be made with reasonable confidence.

According to modern conceptions of seafloor creation, basalts from the axial regions of the mid-ocean ridges should be young, and therefore magnetised in approximately the same direction as the present geomagnetic field. But the few magnetic measurements that have been made on basalts collected irrefutably on the crustal block responsible for the existence of the axial magnetic anomaly have yielded conflicting results¹⁻⁵. Normal polarity has been found in samples from the Reykjanes Ridge¹ and from the Mid-Atlantic Ridge near 45°N (refs 2-4), but mixed or reversed polarities were found in two of six basalt cores drilled within the Rift Valley at the same latitude⁵. The mean magnetic inclination at a site cannot be reliably calculated if the number of satisfactorily reoriented samples is too small or if the direction of magnetisation is erratic within a single pillow or between samples. Although these conditions seem to have been met for samples dredged from 45°N (ref. 4), the mean magnetic inclination is 20° lower than expected.

Three of the samples discussed here (CH 31-DR 1-111; CH 31-DR 2-122; CH 31-DR 4-200) represent material dredged during the RV Jean Charcot CH 31 cruise of 1972 (refs 6 and 7). The remaining six samples (ARP 73-10-02; ARP 74-10-15; ARP 74-11-17; ARP 74-13-24; ARP 74-14-31; and ARP 74-17-40) were collected by a telemanipulated arm operated from the bathyscaphe Archimède during the FAMOUS diving programmes of 1973 and 1974 (refs 8 and 9). The locations of the sampling sites are shown in Fig. 1. Six samples were collected in the axial part of the Rift Valley, the remaining three come from the region of the intersection of the Rift Valley and Transform Fault 'A' near 36°57'N. The age of the samples can be inferred from a variety of methods.

The oxygen isotope ratio for a rock from dredge haul CH 31-DR 1 indicates that the material is very fresh, having suffered no noticeable seawater contamination (G. Pineau, M. Javoy and J. Bottinga, personal communication). Samples from dredge CH 31-DR 2, dated on a relative scale by measurements of the thickness of the palagonite-manganese coating¹⁰ are dated at less than 170,000 yr BP. No age determination is available yet for samples from dredge CH 31-DR 4.

The youngest rock of the set of Archimède samples in the inner floor of the Rift Valley is probably ARP 74-10-15,

Table 1 Magnetic properties of specimens of pillow lavas from the rift valley of the Mid-Atlantic Ridge near 37°N

Location*	Sample number	Specimen	$J_0^\dagger$	MDF	$I_{s0}^\S$	$I_s^\S$	$\theta^\P$	$I_c^{ }$	I_c^{**}
1	CH 31-DR1-111	U (G)	23.4	834	68.7	68.3	2.2	67.2	67.2
		L	220	325	63.8	66.1			
2	CH 31-DR2-122	U	173	243	66.1	65.4	1.5	64.7	64.7
		L (G)	64.5	311	64.2	63.9			
3	CH 31-DR4-200	U (G)	16.9	130	53.2	50.4	3.7	50.0	50.0
		L	109	554	35.2	49.6			
4	ARP 73-10-02 (1)	U	405	181	45.1	43.3	8.9		
		L	170	122	41.3	47.9			
	ARP 73-10-02 (2)	U	288	113	54.7	53.9	3.7	53.7	53.7
		L	171	153	52.5	53.5			
5	ARP 74-10-15 (1)	U (G)	108	495	56.7	55.7	2.3	56.5	56.5
		L	503	226	51.7	57.3			
	ARP 74-10-15 (2)	U	246	300	48.6	55.8	11.2		
		L	455	226	59.9	60.2			
6	ARP 74-11-17 (1)	U (G)	43.5	877	70.1	70.4	0.9	70.7	
		L	348	141	71.4	70.9			
	ARP 74-11-17 (2)	U (G)	33.5	806	69.6	69.2	0.7	69.5	70.5
		L	319	156	69.1	69.7			
	ARP 74-11-17 (3)	U (G)	56.8	849	69.9	70.2	2.5	71.2	
		L	262	170	68.7	72.3			
7	ARP 74-13-24 (1)	U	99.0	444	44.7	43.4	13.8		
		L	67.2	229	23.9	30.7			
	ARP 74-13-24 (2)	U	92.2	474	43.1	46.2	13.5		
		L	62.0	141	29.0	36.7			
	ARP 74-13-24 (3)	U	107	433	44.1	45.1	9.2		
		L	65.8	181	24.3	36.6			
8	ARP 74-14-31 (1)	U (G)	17.7	693	47.4	47.4		47.3	
		L	54.1	523	44.3	47.1			
	ARP 74-14-31 (2)	U (G)	28.3	543	50.9	50.7	4.6	48.7	48.0
		L	78.1	331	42.5	46.7			
	ARP 74-14-31 (3)	U (G)	33.9	639	47.6	49.1	7.4		
		L	49.5	475	49.3	48.2			
9	ARP 74-17-40 (1)	U	106	83	42.1	42.1	1.8	41.7	40.7
		L	99.8	192	25.1	41.2			
	ARP 74-17-40 (2)	U	117	85	58.5	40.0	2.2	39.7	
		L	153	113	41.3	39.3			

* Location refers to numbered sites in Fig. 1. Sample number is eventually followed by core number (in brackets); U, upper specimen; L, lower specimen; G, glass on specimen.

$\dagger J_0$, mean intensity of magnetisation before cleaning (10^{-4} e.m.u. cm $^{-3}$).

$\ddagger$ MDF, median destructive field in peak oersted.

$\S I_{s0}$ and I_s are magnetic inclinations before and after cleaning, respectively.

$\P \theta$, angular deviation between the directions of magnetisations of the two specimens from the same core.

$|| I_c$, mean inclination of core.

$** I_p$ mean inclination of pillow. I_s , θ , I_c and I_p calculated after a.f. demagnetisation.

which exhibited fresh glass on the chilled margin, followed by ARP 74-11-17, which was sampled in a talus pile barren of sediment, and which showed very little preserved glass. Both are olivine basalts^{7,9} representing the youngest eruptives in that section of the Rift Valley, probably emplaced within the past 10,000 yr (refs 10 and 11). The age of sample ARP 73-10-2 is bracketed between 30,000 and 100,000 yr on the basis of these palagonite-manganese coatings¹⁰. Fission-track dating (D. Storzer, personal communication) shows that this sample was formed less than 50,000 yr ago.

The remaining three Archimède samples (ARP 74-13-24; ARP 74-14-31; and ARP 74-17-40) come from the axial portion of Transform Fault 'A' in the region close to its intersection with the Rift Valley. Sample ARP 74-13-24 was taken 4 km east of the axis of the Rift Valley segment on a small north-south ridge which structurally is part of the Rift Valley wall. Its age should be about 270,000–300,000 yr according to spreading rates for the eastern limb of the Rift Valley, calculated from magnetic profiles^{8,12}. Sample ARP 74-14-31, with a rosey surface showing flow direction, has a preserved glassy crust and a palagonite layer overlain by a thin manganese coating. Samples at this site, on the edge of a tension gash associated with the left lateral shear of Transform Fault 'A' (ref. 9), are the youngest specimens collected in the Transform Fault. This estimate is supported by a fission-track age (D. Storzer, personal communication) of less than 45,000 yr. Sample ARP 74-17-40, closest to the accreting plate boundary, has textural and structural features similar to those of rocks determined to be intru-

sives from field evidence. It was sampled on a tectonic scarp which exhibits a layered sequence (possibly a truncated sill) 1.5 km east of the deep axis of the Rift Valley. Its age, inferred from spreading rates, should be less than 100,000 to 113,000 yr (refs 6 and 12). Spreading rate values averaged over the Brunhes Epoch may not necessarily, however, be applicable to an area which may be tectonically and volcanically active. Fission-track ages for this general region are all less than 300,000 yr (D. Storzer, personal communication).

On the combined evidence of fission-track dating, calculated opening rates, palagonite and manganese thickness, and field observations, it seems certain that all the reoriented rocks were emplaced during the Brunhes Epoch, so that a proper scheme of reorientation should reveal that the samples are all normally magnetised. Any negative inclinations could be attributable to eruptives that cooled during the Laschamps (reverse) geomagnetic excursion, which may have occurred between 10,000 and 15,000 yr BP, or during the Blake reverse event (or excursion) with an inferred duration of about 10,000 yr and a mean age close to 110,000 yr BP (see ref. 5).

The partial reorientation of samples from the sea floor is possible first, for cores, assuming that the axis of drilling is vertical (in practice, deviation from the vertical can reach 15°; ref. 5 and, second, for magnetically viscous samples, using various magnetic tests; refs 2, 13 and 14), (these methods should obviously be applied only to rocks whose viscosity has been established; they cannot be used for the samples discussed here because their viscosity index

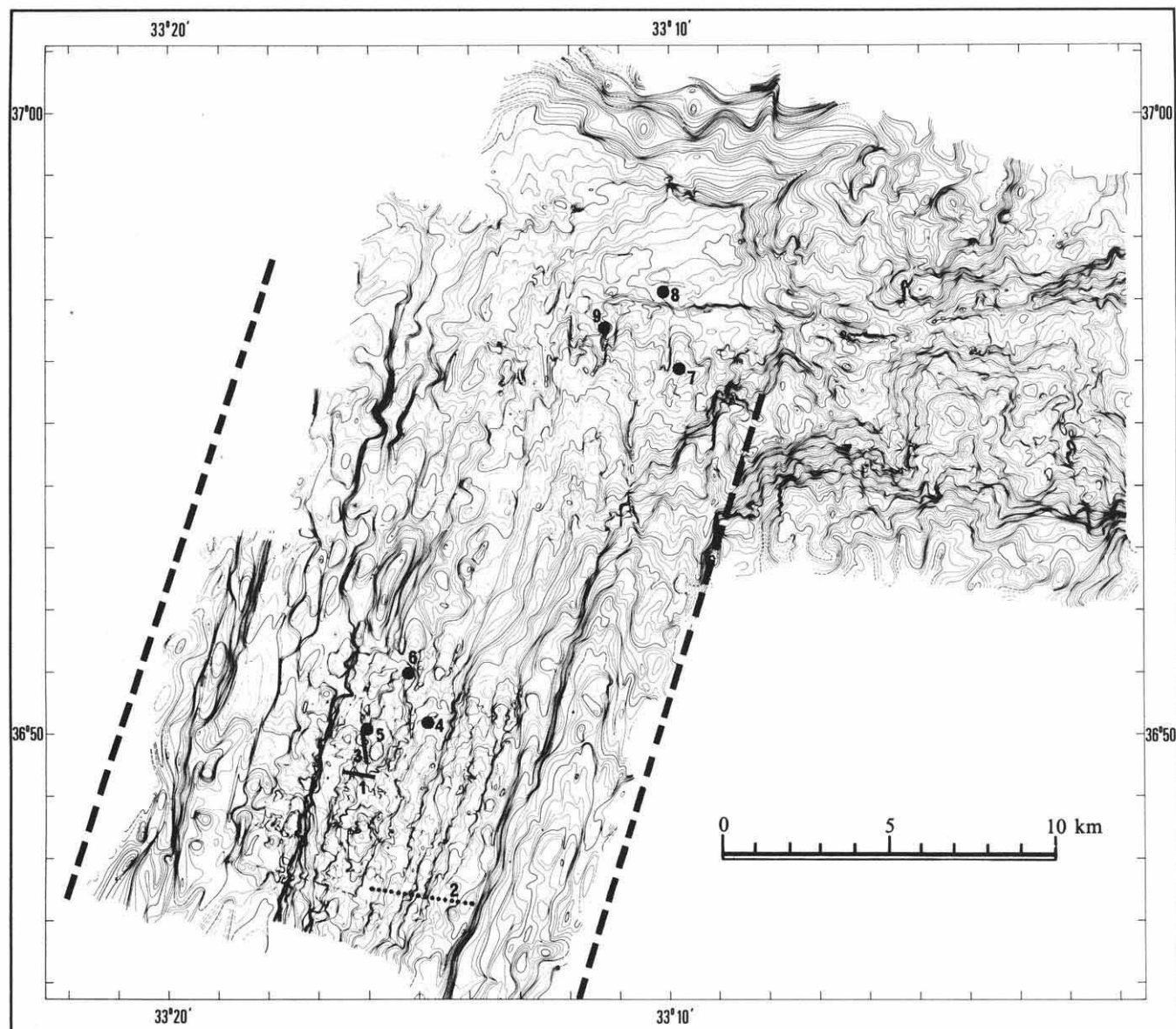


Fig. 1 Bathymetry of parts of the Rift Valley and Transform Fault "A" (21) showing sampling sites (Table 1) and boundaries (heavy dashed lines) of the crust formed during the Brunhes Epoch⁶. The sampling sites are taken from refs 6, 8 and 9. The location of dredge 2 (dotted line) is only indicative. The numbered locations refer to the first entry in Table 1. The Rift Valley sites are all clearly within the Brunhes block and should therefore have normal polarity.

is too small¹⁵); third, for pillow-lavas exhibiting one or more volcanic features considered to be criteria of polarity^{1,4}.

The last method is the only one which gives indications of the orientation of the pillow-lava during cooling because it is independent of possible subsequent changes of position.

De Boer *et al.*¹ have listed four volcanic features thought to be diagnostic of vertical and top: the flattened-oval shape of pillows; the location of necks interconnecting pillows at their bases; stemlike extensions commonly at the bases of pillows; and basal stalactites on the concave side of a crust from lava tunnels or tumuli. Of these, the last seems to be the most reliable (see, for example, ref. 16) but it can give only a polarity and cannot give the vertical because the position of the lava crust with stalactites on the tumulus or tunnel cannot be known *a priori*. In another study⁴, the horizontal was assumed to be parallel to the equatorial plane of the pillow but the assumed error of 20° (ref. 4) is probably too low, because the method relies strongly on questionable conceptions of how pillows form¹⁸, and assumes that the pillow cooled on a horizontal surface. A further possibility, that of using surface glass coating as

a guide to the original orientation, is not reliable because pillows often curl during their formation.

An improved criterion of orientation came from field observations made from the American submersible Alvin in the inner floor of the Rift Valley in the FAMOUS area¹⁷⁻¹⁹. It was noted that, on the tops of lava flows, series of ledges commonly occur along the inner walls of collapsed feeder tubes. The ledges record the various levels at which lava flowed through the tubes¹⁷ much as rings in a bathtub register successive water levels¹⁸. These frozen-in lava levels were observed to be nearly horizontal *in situ*, and they provide a reliable guide to sample orientation for palaeomagnetic studies⁹. The nine samples for which measurements are reported here are the only ones among all the rocks sampled by the French team in the FAMOUS area showing the 'bathtub ring' criterion or a modification of the criterion. Some examples are shown in Figs 2 and 3.

In sample CH 31-DR 4-200 (Fig. 2b), a small flattish ledge occurs near the core of a pillow exhibiting a glassy margin. The upper surface of the ledge is very smooth and contrasts with the overlying, subparallel wall, which has a

rugose texture imposed by the presence of small septae and lava stalactites. The vertical is taken normal to the flattish ledge and the polarity is given by the relationship between the rugose stalactite-bearing surface and the smoother, upward-facing surface. The flattish glassy surface is tilted about 30° from the horizontal.

The angular discrepancy between the glassy surface and the horizontal is more extreme in the case of sample ARP 73-10-02 where the glassy surface is nearly vertical (Fig. 2c). In the interior of the pillow a series of elongate cavities with smooth flattish floors and more irregular roofs gives a good indication of the vertical at the time of cooling.

In sample ARP 74-11-17 (Fig. 2d), the glassy surface is on top and nearly horizontal. The vertical is taken to be normal to a line of small, elongate, asymmetric cavities. The true vertical could deviate slightly, because the cavities seem to be *en échelon* (Fig. 3) and the vertical axis could have been taken perpendicular to the parallel long axes of the cavities. The polarity is not, however, in doubt and is given by both the asymmetry of the cavities and by the lava stalactites on a large surface (Fig. 3).

Sample ARP 74-14-31 shows a superposition of two, approximately horizontal, ropey lava surfaces (Fig. 2a). A confirmation of the field determination is provided by an elongate cavity below the upper ropey crust and by well developed stalactites on the bottom of the sample.

Suitable samples for reorientation could not be found in the collection made from the diving saucer Cyana, because of their small size. Similarly, only the very large dredge samples could be used with any confidence.

One to three vertical cores, about 10 cm long, were drilled through each of the nine samples of reoriented pillow lava basalts. The upper and the lower parts of each core were sawn flat, giving two specimens of about 30 g. All 36 specimens were submitted to magnetic study, including alternating field treatment up to 850 oersted (Table 1). In several cases, the intensities of magnetisation and the median destructive field (MDF) were quite different from one specimen to another, even within a single core. In particular, the glassy parts of a sample are systematically much less magnetised than non-glassy parts. The very high coercivities shown by

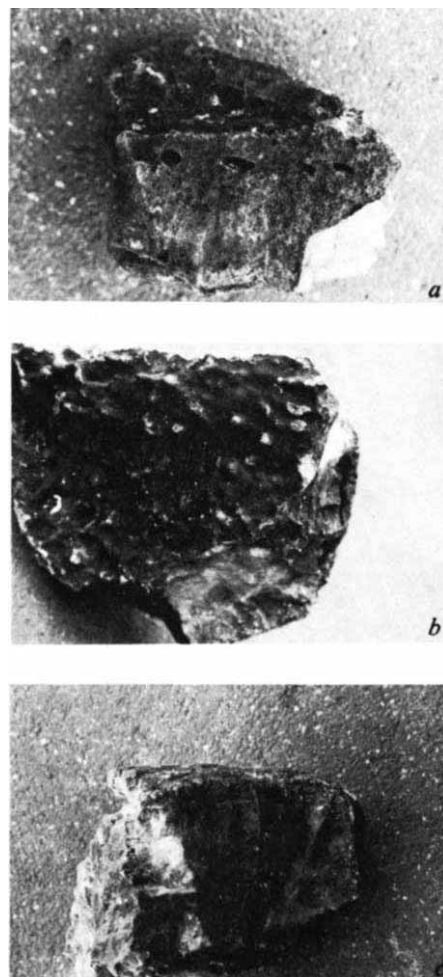
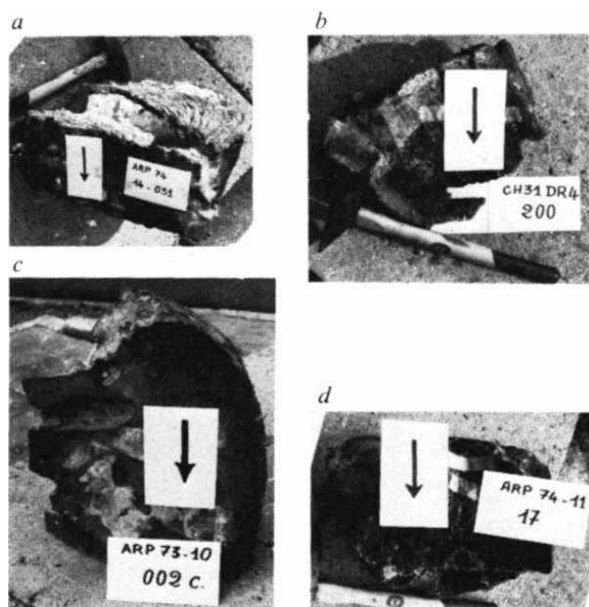


Fig. 3 a, Close-up of cavity alignment in sample ARP 74-11-17 (Fig. 2 d); b, stalactite-bearing surface (vertical-upward into picture) at the base of ropey lava ARP 74-14-031 (shown in Fig. 2); c, close-up of horizontal cavity below upper ropey surface of sample ARP 74-14-031 (Fig. 2a).

Fig. 2 Four selected pillow lavas displaying characteristic features for reorientation (see text). Arrows, vertical-downward at the time of cooling. a, ARP 74-14-031; b, CH 31-DR-4-200; c, ARP 73-10-02c; d, ARP 74-11-17.



the glassy portions of the samples probably reflect in part the smaller grain size. As previously pointed out²² the between-specimen variability indicates that large samples (or the averaging of results from several specimens from each pillow) are generally required to obtain significant data on the intensities of magnetisation of submarine extrusives.

All of the specimens have a positive magnetic inclination. The alternating field treatment induced small changes in direction, probably by the removal of weak parasitic magnetisations acquired during drilling. Because the samples are not viscous, no significance can be attached to the sign of the inclination change during cleaning. In some cases, the directions of magnetisation after cleaning remain significantly different for the upper and the lower specimen from a single core. In particular, the upper part of pillow ARP 74-13-24 is characterised by a magnetic inclination 10 – 15° higher than that of the lower part of the pillow. This may indicate variations in space of the geomagnetic field at the site of cooling, because of disturbances caused by the neighbouring lavas. The large inclination found for sample ARP 74-11-17 may be real and result from secular variation. Alternatively, it may be the result of a small error in the orientation, as discussed above.

In calculating the mean inclination of a pillow we have rejected data obtained from cores exhibiting a deviation larger than 5° in their directions of magnetisation (after cleaning) between the upper and the lower part of the pillow.

The overall mean magnetic inclination, calculated from the mean values for eight pillow basalts, is equal to 56° . It corresponds exactly with the inclination of the present geomagnetic field (International Geomagnetic Reference Field, 1965), which is the same as the inclination of an axial dipole field. At two drill sites of DSDP leg 37, west of the FAMOUS area, natural remanent inclinations were also found to be close to the dipole value for the area and to match this local anomaly sense²⁰ although anomalous shallow inclinations were found at a third site.

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Dune trend and the Ekman Spiral

FRICTION at the Earth's surface induces deflection of the ground wind from the geostrophic direction, the progressive change with height being known as the 'Ekman Spiral'. As the roughness of the surface increases, this deflection becomes further to the left in the Northern Hemisphere and further right in the Southern; the differences between extensive water or grass-covered areas and nearby rough surfaces, such as rooftops, are commonly 15° (ref. 1). The evidence presented here shows that dune fields, many of which have rougher surfaces than neighbouring areas, and within which there may be differences in roughness between dune types, show evidence of such deflections.

Deflection is most apparent in coastal dunes, probably because wind recording stations are relatively common on coasts, and because the important onshore winds encounter a marked contrast between the surface roughness of the sea and that of the dunes.

Landsberg² examined twelve parabolic dune fields on the coasts of Britain and Denmark; in eight of these the dune trends are to the left of the resultant of sand movement at the nearest wind recording station (that is, the average wind direction weighted by its ability to blow sand), and in two there is little difference. The site at Burghead in Scotland

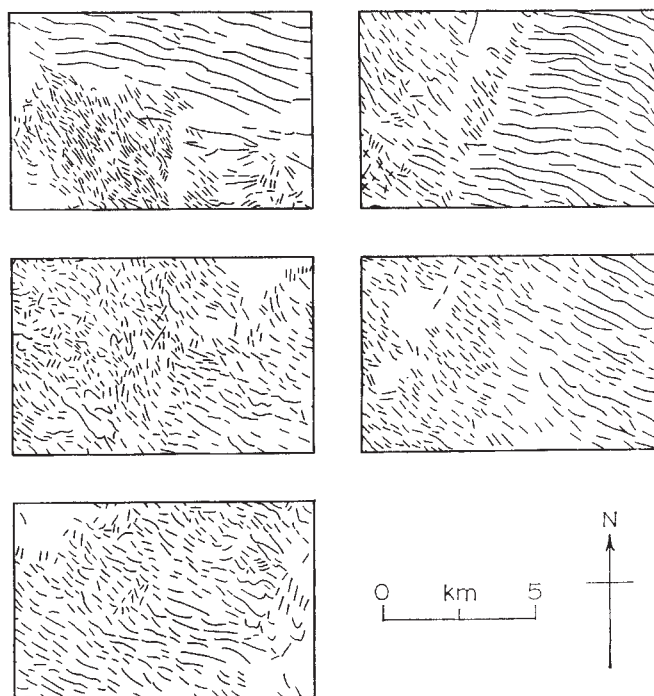


Fig. 1 Patterns of transverse-type dunes in the fixed Qoz sand dunes of Central Sudan. Each line represents a dune crest; the winds came from a northerly direction. The wind at right angles to the longer wavelength dunes is always to the left of that normal to those with lower wavelengths.

shows too marked a leftward deflection to fit with Landsberg's explanation that it is the result of the occlusion of landward winds at the recording station. The sites which show a rightward deflection do so when compared with very distant recording stations. In Gower, South Wales, Harris³, like Landsberg, invoked sheltering to explain the discrepancy between the trend of the sand moving resultant and the dunes, but here too there is a distinct leftward deflection of the dunes.

In the dune fields of the Oregon coast there is a consistent difference between the trend of the dominant winds and of the dunes: lines perpendicular to nearshore transverse dunes have an orientation from 1.5° to 22° to the left of the dominant NNW winds which are thought to form them⁴. One recording station, North Bend, which is some km inland from dune country, shows that the dominant wind has swung to the left (it is more westward than at the coastal stations). Cooper's⁵ data for the California coast are sparser than those for Oregon, but again nearshore dunes set consistently to the left of the July north-westerlies which are apparently responsible for them.

In the Southern Hemisphere the trend of parabolic U-dunes on the eastern, western and southern coasts of King Island, Tasmania is almost everywhere to the right of the sand moving resultant of the onshore winds⁶.

Turning now to the internal evidence of the dune patterns themselves, it can be seen on both Landsberg's² and Jennings's⁶ maps of parabolic U-dunes that the deflections consistently become more pronounced inland, presumably in response to increased surface roughness. In Oregon the direction at right angles to the mean trend of low transverse dunes is skewed well to the left of the trend of more longitudinal near-shore lee features, which presumably have lower surface roughness⁴. Further inland, the leftward swing becomes more pronounced in the long seif-like dune ridges. Cooper related the trend of these to the yearly resultant of sand moving winds (although he did not weight these for sand movement), but the wind records at North Bend, mentioned above, support the idea that the dominant

winds veer to parallel the ridges inland. On the California coast, all the transverse ridges of Flandrian age shown on Cooper's maps³ imply winds to the left of the trend of the nearshore longitudinal lee features. On the coast of Israel, transverse dunes again indicate a wind to the left of closely associated longitudinal features (a fact which Rim explained as due to an asymmetry in the sand supply)⁷.

Desert dunes are not as likely to show such clear evidence of discordance as coastal dunes because wind recording stations are very sparse and are themselves often in terrain as rough or rougher than dune surfaces, and also wind regimes are more complex, since they include relevant winds from many more directions than the simple onshore winds responsible for most coastal dune forms. In spite of these difficulties, many inconsistencies of trend can be explained with the Ekman Spiral. It is found that, as with coastal dunes, the rougher-surfaced transverse dunes are commonly skewed more than longitudinal dunes.

Sprigg⁸ suggested that there had been a 4°–5° southward shift in windbelts since the time of dune formation in central Australia, because the sand ridges trend to the right of the wind, but a simple application of the Ekman Spiral shows that if the wind recording stations were in smoother terrain, the dunes should indeed show this trend. The common rightward asymmetry (though not the orientation) of these ridges has been explained with the Spiral by Mabbutt and others⁹. Brookfield¹⁰ found no simple pattern of deviations, but did note that well developed ridges in the Simpson Desert were aligned to the right of the resultant sand movement, and more recently Twidale's¹¹ maps shows that, in most cases, transverse-type dunes in the same desert would be perpendicular to winds which are to the right of those which parallel the sand ridges.

In the Northern Hemisphere, Clos Arceduc¹² has repeatedly used air photographs to show that long seif or 'slouk' dunes cannot be longitudinal to the winds flowing over nearby barchans. Most of his seifs are to the right of this putative wind, a fact which could be explained if the wind deviated to the left of the geostrophic direction as it passes over the rougher surface of the transverse barchans. Indeed over groups of barchans the deviation is more marked than over single ones (compare numbers 31 and 34 with 37 on Fig. 2 of ref. 12). In the Ténéré Desert, Warren¹³ showed that the wind at right angles to transverse zibar would be 14° to the left of one parallel to closely associated seif dunes, and nearby, near the oasis of Bilma, a small dune field of seifs and barchans again indicates that the wind at right angles to the barchans would be to the left of the main trend¹⁴.

Hack's¹⁵ maps of the Navajo county of the south-western USA show yet again that transverse dunes would be perpendicular to winds which are to the left of long straight seif-like dunes. Near the Salton Sea, Long and Sharp¹⁶, using wind records from an admittedly distant station, noted that barchans had moved to the left of the dominant winds, during both a 7-yr and a 15-yr period.

The evidence of desert dunes is not, however, entirely in favour of this Ekman Spiral hypothesis. In a pattern in Libya which was very similar to the one in the Ténéré discussed above, McKee and Tibbetts¹⁷, found that transverse zibar indicated a wind to the right of the one which would parallel the local seifs, and further south in southern Niger, Grove and Warren¹⁸, found megadune patterns east of Termit and south-west of Agadem in which the transverse features are again apparently aligned to a wind to the right of the one parallel to the longitudinal ones.

The morphology of Pleistocene-fixed dune sands is difficult to interpret in the absence of wind data for the period of their formation, but there is some evidence of divergences over different dune types. In Central Sudan¹⁹ transverse dunes with long wavelengths (rougher surfaces) are invariably at right angles to winds which would have been to

the left of those perpendicular to juxtaposed transverse dunes of lower wavelengths (less rough) (Fig. 1). These large areas of uniform roughness may offer an opportunity to measure the value of surface roughness parameter.

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Electrical properties of magnetisable liquids

RECENTLY developed magnetisable liquids comprising colloidal suspensions of soft ferromagnetic particles have attracted considerable scientific and technological interest^{1–3}. The ferromagnetic grains are coated with a special film which prevents them from clustering together and maintains a true colloidal dispersion⁴. We have investigated the sensitivity of their electrical properties to magnetic fields and found some new 'magneto-electric' effects. An interpretation of these observations is suggested.

Conductance and capacitance measurements of a commercial magnetisable liquid (Ferrofluid water base, A01, 200 gauss saturation, Ferrofluidics Corporation) were made using a Metrohm conductivity probe (type E A 608). This is of a standard type for conductivity measurements and consists of a pair of parallel electrode plates coated with porous platinum. The conductivity of the fluid between the plates σ is obtained from the measured conductance S through the geometrical factor c , where $c \approx \sigma/S \text{ cm}^{-1}$.

For the probe we used the value of c was stated by the manufacturers to be $c = 0.83 \text{ cm}^{-1}$. Capacitance measurements were made with the same probe. All the a.c. measurements were made on a General Radio type 1650-B bridge operated with an external signal generator.

The frequency dependence of conductivity and capacitance for the liquid is shown in Fig. 1. Note that, although the conductivity is approximately constant over the frequency range, the capacitance varies as f^{-2} over most of the range. At low frequencies the measured capacitance becomes very large ($\approx 1 \mu\text{F}$).

Figure 2 represents the d.c. characteristic of the magnetisable liquid. As in the case of the a.c. measurements, the geometrical factor c may be used in order to derive the conductivity from the measurements. Below 5 V, the voltage corresponding to a steady current takes some time to reach a steady value, indicating that not only is the resistance larger than the measured a.c. values, but the capacitance is also significantly larger than those found with a.c. down to 40 Hz. The time constant of 0.5 V was $\sim 3 \text{ min}$.

Similar experiments were performed in the presence of magnetic fields, to determine the effect of applied homogeneous and inhomogeneous fields on the electrical properties of the liquid. A solenoid coil wound round the entire column of liquid was

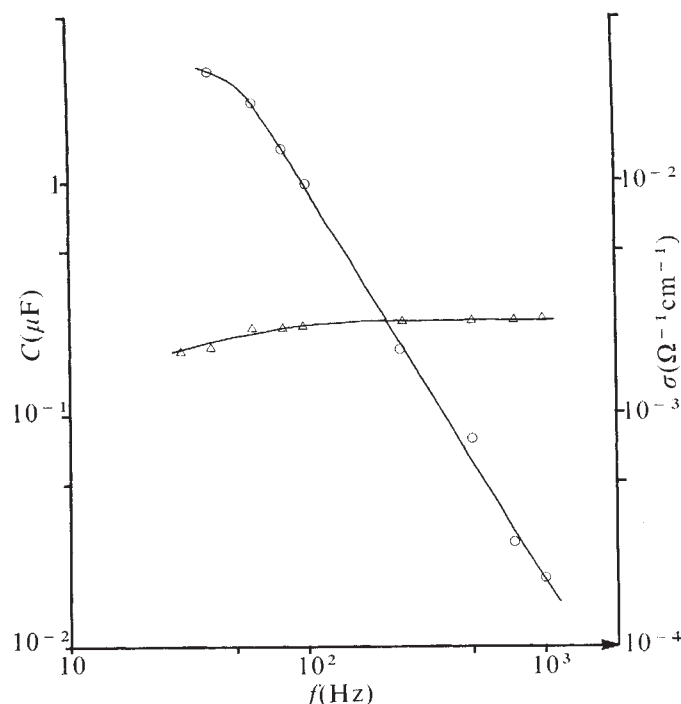
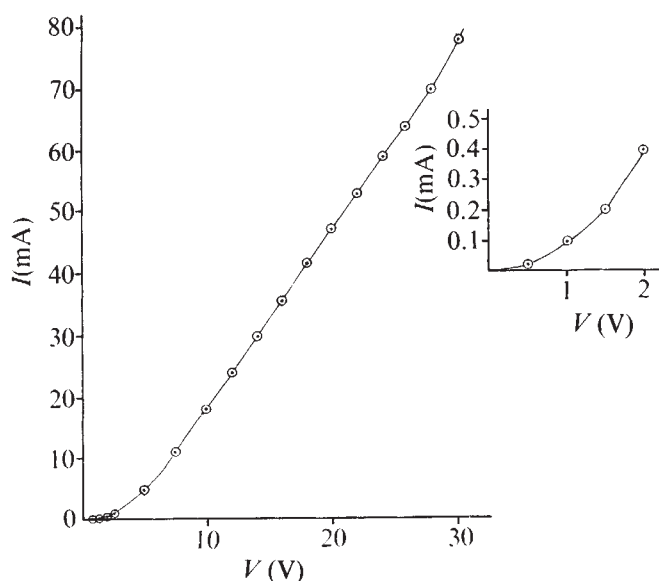


Fig. 1 Measured capacitance (C , $\circ$) and electrical conductivity (σ , Δ) as a function of frequency (f) of the magnetisable liquid.

used as the source of a relatively homogeneous field. The relative permeability of the liquid was found to be ~ 1.2 in other measurements (not reported here). It may, therefore, be assumed that the liquid and probe hardly influence the homogeneity of the applied magnetic field. For inhomogeneous fields, a small horseshoe magnet was fitted around the measurement tube, and produced a magnetic field in the liquid varying from zero to 600 gauss.

Homogeneous fields were found to have a negligible effect on the resistance of the magnetisable liquid, but large changes in the current could be effected in the low-voltage region (< 5 V) by appropriately aligning the position of the horseshoe magnet. Thus, for example, at 0.5 V the current increased by $2 \mu\text{A}$ from its value of $40 \mu\text{A}$ in the absence of a field, representing a 5% overall change.

Fig. 2 d.c. Voltage-current characteristics of the magnetisable liquid.



It was also established that a homogeneous field produced changes in the capacitance as measured on the a.c. bridge. At 750 Hz, for example, the change in capacitance as a function of magnetic field applied perpendicularly to the conduction path is shown in Fig. 3. At 270 gauss, the increase in capacitance is $\sim 0.3\%$. A magneto-resistance effect is also noted but the effect is considerably weaker.

Figure 1 suggests that the unusually large measured capacitances are not bulk values for the liquid but arise from a polarised layer on the surface of the porous electrodes, (or an analogous effect in the colloid), the resistance seems to represent a bulk property. Accordingly the specific resistivity (or conductivity) of the liquid may be estimated, whereas no simple derivation of the dielectric constant can be obtained from the capacitance measurement.

If the liquid is represented in a simplified model, with the electric path between the two electrodes having a resistance r and series capacitance C_s , then the admittance across the probe is

$$Y_s = \frac{1}{r + (1/j\omega C_s)} = \frac{r - (1/\omega C_s)}{r^2 + (1/\omega^2 C_s^2)} \quad (1)$$

The General Radio Bridge, however, measures a capacitance C as if it were in parallel with a resistance R . In such a configuration, the admittance would be

$$Y_p = \frac{1}{R} + j\omega C_p \quad (2)$$

Comparing the real and imaginary parts of equations (1) and (2),

$$\frac{1}{R} = \frac{\omega^2 C_s^2 r}{\omega^2 r^2 C_s^2 + 1} \quad (3a)$$

and

$$C_p = \frac{C_s}{1 + \omega^2 r^2 C_s^2} \quad (3b)$$

at high frequencies such that

$$\omega^2 r^2 C_s^2 \gg 1$$

$$R \approx r = \frac{1}{S} \text{ (constant)} \quad (4a)$$

and

$$C_p \approx \frac{1}{\omega^2 r^2 C_s} \quad (4b)$$

Equations (4a) and (4b) give a satisfactory description of the experimental results. Above 80 Hz, the measured conductivity S remains almost constant, while the capacitance varies as ω^{-2} (or f^{-2}).

Information about the characteristic dielectric properties of the liquid are not easily obtained from the capacitance measurements because of the complex dependence of the polarisable layer on the specific nature of the electrodes used, and it was found that different electrodes yield different values of capacitance. It is hoped that a better understanding of the polarisable layer or its equivalence will be gained in future investigations.

Support for the above model is provided by the voltage-current characteristic. The large time constants and correspondingly large resistances observed at low voltages indicate high values of the layer capacitance. When the voltage increases, however, there seems to be a gradual breakdown effect, and the measured d.c. resistance approaches its a.c. value of 330Ω . Under a.c. conditions the proposed layer behaves as a coupling capacitance C_s according to equation (1), and therefore a similar

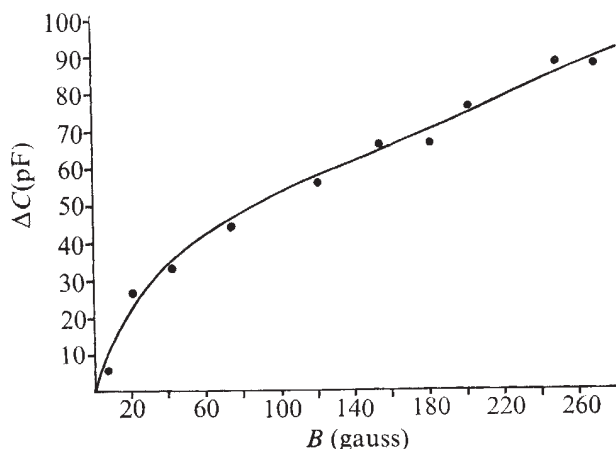


Fig. 3 Increase of capacitance (ΔC) as a function of applied magnetic field B for the magnetisable liquid.

bulk resistance is measured. The a.c. measurements of capacitance made use of small signals and consequently no breakdown effect was encountered.

The cross effects of magnetic fields on the voltage-current characteristics are like the capacitance attributed here to the proposed polarised layer. This is consistent with the observation that the capacitance changes more strongly with magnetic field than does the conductivity in the a.c. measurements. Moreover, at high voltages the influence of the magnetic field on the d.c. measurements drops to zero because of the presumed breakdown of the layer. It is proposed that inhomogeneous fields exert a stronger influence on the voltage-current characteristic at low voltages than do homogeneous fields because they can more easily produce irregularities in the layer which might thereby be locally 'punctured'.

Different electrodes and ferrofluids might increase the magneto-electric effect. It is believed that this phenomenon is affected by the saturation magnetisation of the liquid, as well as by the solvent and dilution of the colloid used. If the effect can be sufficiently enhanced, possible applications may be found in energy conversion and signal processing. From a theoretical viewpoint, we believe that the observed phenomena in magnetisable liquids provide a new means of probing layer effects in colloids and might help provide further insight into the electrical behaviour of colloids in general.

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Search for new silver-ion solid electrolytes for use in batteries

THE α -phase of AgI, which is stable above 146 °C, has a silver-ion conductivity of $1 \Omega^{-1} \text{ cm}^{-1}$ at 150 °C (ref. 1), which would make it a suitable solid electrolyte for high temperature batteries. The room-temperature conductivity of the stable phase of AgI is disappointingly low ($10^{-6} \Omega^{-1} \text{ cm}^{-1}$) and although samples with conductivities approaching $3 \times 10^{-4} \Omega^{-1} \text{ cm}^{-1}$

can be prepared², such values are insufficient to make it of use as an electrolyte in solid state batteries at room temperature. There is, however, considerable interest in producing solid electrolytes, based on AgI combined with organic cation iodides to give high silver-ion conductivity at room temperature⁴⁻⁹. We report here on the conductivities of representatives of three hitherto unexamined classes of compounds, AgI-selenonium iodide, AgI-tellurium iodide and AgI-bis-sulphonium diiodide.

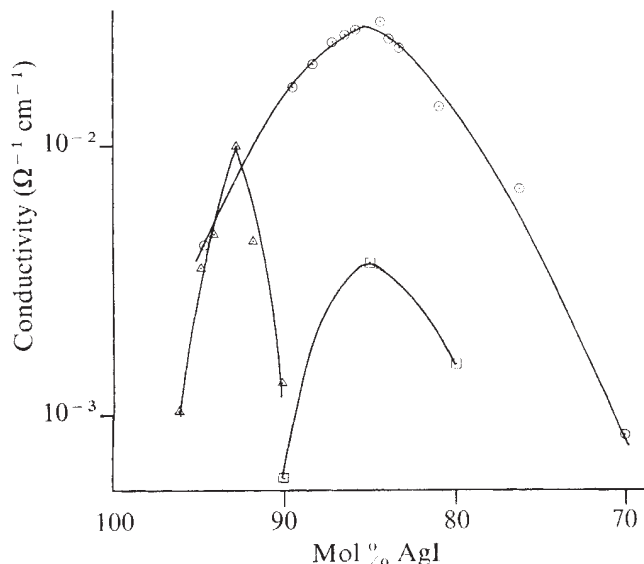
As a representative of the AgI-SeI system, $(\text{CH}_3)_3\text{SeI}$, prepared from $(\text{CH}_3)_2\text{Se}$ and CH_3I (ref. 10) was mixed with varying amounts of AgI to produce samples containing 90, 85 and 80 mol % AgI. The powdered samples were suspended in distilled water and heated to $90 \pm 5^\circ \text{C}$ for a total of 16 h. The solid product was filtered off and washed with ethanol and dry ether, before being pressed as a three-layer pellet, the outer layers of which were silver powder mixed with powdered electrolyte. The conductivities for each of the three samples at this stage were $< 10^{-5} \Omega^{-1} \text{ cm}^{-1}$ at 21 °C. The pellets were then heated in nitrogen to $140 \pm 2^\circ \text{C}$ for 9 h, after which the conductivities at 21 °C reached the values shown in Fig. 1. For comparison, we show also results for AgI- $(\text{CH}_3)_3\text{SI}$. The conductivity for the Se-containing electrolyte was $4 \times 10^{-3} \Omega^{-1} \text{ cm}^{-1}$ at 85 mol % AgI, compared with $2.7 \times 10^{-2} \Omega^{-1} \text{ cm}^{-1}$ for the S-containing electrolyte at the same mol %. A ten-fold deterioration in conductivity was observed over a period of a few weeks, which may have been caused by the presence of moisture¹¹.

The loss of conductivity makes the AgI- $(\text{CH}_3)_3\text{SeI}$ system unsuitable for battery use, although other Se-containing systems may be more suitable. The first member of the AgI-TeI system that we have studied is $\text{Ag}_{5.5}(\text{CH}_3)_3\text{TeI}_{6.5}$ which was prepared by grinding together appropriate amounts of $(\text{CH}_3)_3\text{TeI}$, prepared from $(\text{CH}_3)_2\text{Te}$ and CH_3I (ref. 10), and AgI, pressing into a three-layer pellet and heating to $140 \pm 10^\circ \text{C}$ for 7 h. The conductivity was $< 10^{-5} \Omega^{-1} \text{ cm}^{-1}$, making it unsuitable for battery use.

The relatively poor properties of these Se- and Te-containing electrolytes, when taken in conjunction with their high toxicities and the consequent difficulties that arise in handling them indicate that such systems are unlikely to be exploited commercially for some considerable time.

We have found, however, that the performance of bis-sulphonium diiodides is altogether more encouraging. 1,3-bis(dimethylsulphonium)propane diiodide was prepared by the

Fig. 1 Conductivities of solid electrolyte systems incorporating AgI and (a) $(\text{CH}_3)_3\text{SeI}$ ($\square$); (b) $(\text{CH}_3)_3\text{SI}$ ($\circ$); (c) $(\text{CH}_3)_2\text{S}^+(\text{CH}_2)_3\text{S}^+(\text{CH}_3)_2 2\text{I}^-$ ($\triangle$) as a function of mol % AgI.



method of Protiva, Jilek and Exner¹², ground up with the correct amount of AgI and pressed into three-layer pellets, the outer layers comprising a mixture of silver powder and powdered electrolyte. Pellets containing 96, 95, 94, 93, 92 and 90 mol % AgI were prepared in this way, these proportions being selected because the conductivity maximum for a dication would be expected to be within this region, by analogy with the dimethonium results⁴. The pellets were heated in nitrogen at $120 \pm 2^\circ\text{C}$ for 66 h, after which their conductivities at 21°C were measured. The values are presented in the Fig. 1. The conductivity is $1.0 \times 10^{-2} \Omega^{-1} \text{ cm}^{-1}$ for 93 mol % AgI–7 mol % $(\text{CH}_3)_2\text{S}^+(\text{CH}_2)_3\text{S}^+(\text{CH}_3)_2 2\text{I}^-$, which remained constant over a period of several weeks. This stability, which contrasts with the results for Se- and Te-containing electrolytes, makes this system a plausible choice for use in a solid state cell. Conductivity results for the systems AgI– $\text{R}_2\text{S}^+(\text{CH}_2)_m\text{S}^+\text{R}_2 2\text{I}^-$ and AgI– $(\text{CH}_2)_n\text{S}^+(\text{CH}_2)_m\text{S}^+(\text{CH}_2)_n 2\text{I}^-$, and their use in solid-state electrochemical cells will be reported later.

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Fossil hominid femora and the evolution of walking

THE evolutionary origins of human bipedalisms are still poorly understood, but recent discoveries of fossil hominid femora in Africa reveal some interesting details. Especially important are several new fossils discovered by the expedition led by R. E. F. Leakey to East Rudolf, Kenya¹⁻⁹. The purpose of this paper is to find the morphological and functional affinities among higher primates of these and other fossil femora in the hope of revealing more about the nature of early hominid locomotion.

The hominid fossils analysed here include two from Swartkrans, South Africa (SK 82 and SK 97), discovered in 1949 by Broom and Robinson¹⁰, dated variably between 1.5 and 3 Myr¹¹⁻¹⁵, and classified as *Paranthropus* or *Australopithecus robustus*; and three from east of Lake Rudolf discovered by R. E. F. Leakey's expedition in 1972³⁻⁹ and classified as *Australopithecus* sp. indet. (KNM-ER 1503, dated between 1.8 and 2.6 Myr) and *Homo* sp. indet. (KNM-ER 1472 and 1481c, both dated between 1.6 and 3 Myr)¹⁶.

The comparative sample consists of either the left or right femur from 57 *Homo sapiens*, 42 *Pan troglodytes*, 16 *Pan paniscus*, 66 *Gorilla gorilla*, and 34 *Pongo pygmaeus*.

Ten measurements are used to represent the shape of the

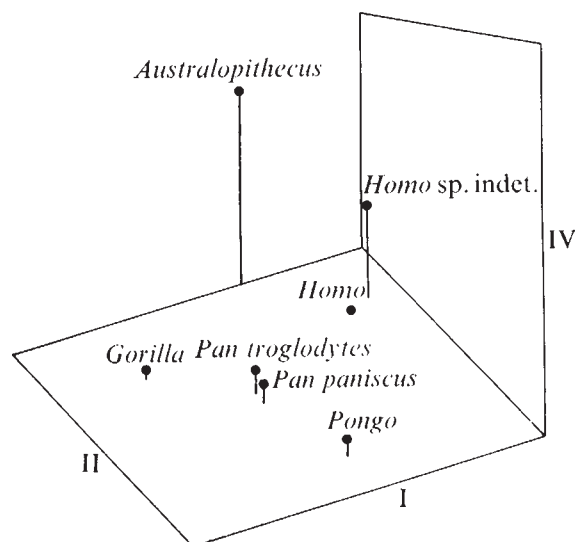
proximal femur. These are the vertical head diameter, vertical neck diameter, anteroposterior neck diameter, transverse shaft diameter, anteroposterior shaft diameter, the projected distance from the centre of the head to the lateral surface of the greater trochanter measured perpendicular to the shaft axis, neck length, two measurements from the inferior border of the lesser trochanter: one to the superior surface of the neck, and one to the centre of the head, and the projection of the greater trochanter above the neck. A full description is given elsewhere¹⁷.

These data were analysed using canonical variates analysis¹⁸. The measurements are first adjusted for possible allometric effects, using average within-taxon allometry coefficients between each measurement and a standard size variable¹⁹⁻²¹. They are then converted to ratios of the size reference variable for each specimen, producing dimensionless shape variables which are relatively independent of factors of size. Canonical analysis is performed by entering each taxon as a separate group. All of the hominid fossils are entered as a single group in the original calculations.

Figure 1 displays the results by plotting the first, second and fourth canonical variates, which together account for 82.9% of the total variance (47.3%, 30.9%, and 4.7% for axes 1, 2, and 4, respectively). The third canonical variate is not plotted because it merely acts to separate *Pan* from the other hominoids. The fossil hominid femora are quite variable, which might be expected considering the changes in locomotor adaptation in human evolution and their possible time span. The three femora that have been classified³⁻¹⁰ as *Australopithecus* (SK 82, SK 97, and KNM-ER 1503) are separated from *Homo sapiens*. The two fossils classified as *Homo* (KNM-ER 1472 and 1481c) are closer to modern *Homo sapiens*.

On the first canonical variate all of the hominids are separated from the rest of the primates. The variables with the highest correlation with this variate include the neck length and the distance that the greater trochanter projects above the neck. The neck is very long in all of the fossil femora and moderately long in modern *Homo*. The human greater trochanter is lower than in the other hominoids, but in all of the early hominids the greater trochanter barely projects at all. Presumably the differences in the projection of the greater trochanter reflect differences in the function of the gluteus medius, gluteus minimus, and piriformis muscles which attach on the greater trochanter. The two glutei muscles act primarily as abductors in bipedal

Fig. 1 Distribution of group centroids and fossils on the first, second, and fourth canonical variates which account for 82.9% of the total variance.



hominids—their pull is essential to the lateral support system in the human hip²². The length of the femoral neck is another feature which is associated with the abductor mechanism of the bipedal hominid hip, since the length of the neck is related to the length of the abductor lever arm. The very long femoral neck of the early hominids may imply that they had mechanically efficient lateral support mechanisms in their hips²³.

The second canonical variate maximises the separation of *Pongo* from the other hominoids, and the variables with high correlations with this axis are those which describe the unique morphology of the orangutan hip. The third variate separates the two species of *Pan* from the rest of the primates. It is on the fourth variate that the fossil hominid femora differ significantly from modern *Homo*. Traits most closely associated with this axis are the same as those associated with the first variate, except the head size is also heavily weighted. In the three hominid fossils classified as *Australopithecus* (KNM-ER 1503, SK 82, SK 97) the head diameters are uniquely small, but in the two classified as *Homo* (KNM-ER 1481c and 1472) they are more similar to extant hominids. As a consequence of this resemblance and others, the two early *Homo* femora lie nearer to the modern *Homo* group on the fourth canonical axis, with the australopithecine femora in an isolated position.

Other axes account for the small residual variances, but offer no other useful interpretation.

Figure 2 compares the relative size of each dimension in the two most complete fossil hominids (KNM-ER 1481c and SK 97) and *Homo sapiens*. The significant fact revealed by this figure is the similarity between the two hominid fossils in many traits and how different they are in the same way from *Homo sapiens*. The only conspicuous differences between the two fossils are in their relative head size and relative anterior-posterior shaft thickness. This result contrasts with the preliminary visual assessment and many univariate comparisons concerning KNM-ER 1481c.

These results can be related to what is known of australopithecine hip-joint mechanics. Although these fossil hominids were bipedal, their pelvic girdles are morphologically different from modern humans in having laterally splayed iliac blades, small acetabulae, and long femoral necks. Lovejoy *et al.*²³ give a biochemical explanation of these differences suggesting that the evolutionary changes between the hip of the australopithecines and *Homo sapiens* is a result of encephalisation: the increase in cranial size in human evolution meant a concomitant increase in size in the birth canal. The intermediate position of the two East Rudolf femora classified as *Homo* could be related

to the intermediate cranial capacity apparent in that population.

Although the present study deals with only one half of a joint and not with a total functional unit, the proximal femur is an integral part of hominid locomotion. Its morphology is highly correlated with that of the pelvis and other parts of the hindlimb. Unfortunately, there are no published reports of complete pelves associated with femora in the early hominid fossil collections except for Sts 14 from Sterkfontein, South Africa, but a large part of its proximal femur is missing, including the head and most of the neck.

The findings do support the idea expressed by Leakey and others³⁻⁹ that there were at least two distinctive forms of hominids with one more closely related to the later hominids than the other. The distinctively long neck of all of the early hominids indicates that the abductor lever arm was probably long which would yield a favourable bio-mechanical arrangement for the lateral support system in the hip which is necessary for human walking. The differences between the fossils classified as *Homo* and *Australopithecus* may imply that the hip joint mechanics were different. A major morphological difference is the size of the femoral head. If Lovejoy *et al.*²³ are correct in their assessment of early hominid hip biomechanics, the large femoral heads might be a result of a widened birth canal. Whether or not there was more than one kind of gait among the early hominids is still uncertain.

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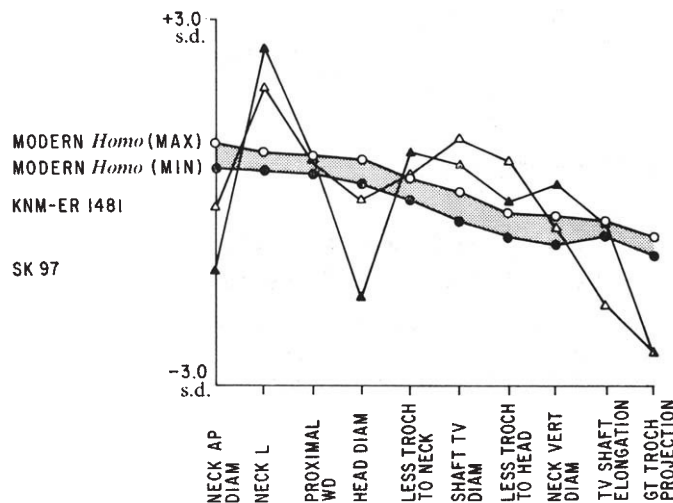
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Fig. 2 Comparison of shape variables between *Homo sapiens* and the two most complete hominid proximal femora (KNM-ER 1481c and SK 97).



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Hypothesis for origin of planktonic patchiness

ALTHOUGH environmental heterogeneity can contribute to the observed patchiness in oceanic planktonic populations, biological interactions between phytoplankton and herbivorous copepods can lead to similar patterns. The phenomenon of spontaneous pattern generation even in a homogeneous environment through the interplay of reaction and movement is a very general property of the class of mathematical 'reaction-diffusion' equations used to model such systems, and there are several ways in which such patterns may occur. The result may be either spatiotemporal patterns¹ or time-independent spatial patterns. Usually one thinks of diffusion as damping inhomogeneities, and a hypothesis put forward by Steele² essentially relies on a balance reached between the dehomogenising aspects of local interaction and the homogenising influence of diffusion to produce pattern. On the other hand, it has also been suggested³⁻⁶ that in some conditions diffusion can destabilise an otherwise stable interaction (in the manner originally suggested by Turing⁷) to produce pattern. We have explored⁵ this further in a nonlinear analysis that describes the final stages of pattern formation, and the resultant calculations provide some insights into the spatial scale of patterns which might arise in this way.

The results that we describe depend on two hypothesised properties of the full equations: an autocatalytic effect in phytoplankton density which is attributed to a reduced efficiency of herbivory as phytoplankton density increases (a Holling⁸ Type 2 functional response), and differential dispersal rates which favour higher herbivore motility. Both assumptions may be justified on theoretical grounds (the work of Holling for the first, and the greater mobility of herbivores by comparison with phytoplankton for the second), but the data are at present insufficient to provide the real test.

The relevant equations⁵ thus take the form

$$\partial P/\partial t = aP + eP^2 - bPH + \nabla \cdot (\mu \nabla P)$$

$$\partial H/\partial t = cPH - dH^2 + \nabla \cdot (\nu \nabla H)$$

where P is phytoplankton density, H herbivore density, t time, and μ and ν are species-specific diffusion coefficients. a , b , c , d and e are all assumed positive. The assumption of passive diffusion is made for simplicity only; more complicated movement patterns can also lead to diffusive instability.

Assuming homogeneity of dispersal rates and restricting attention to one space dimension, we obtain the modified equations

$$\partial P/\partial t = aP + eP^2 - bPH + \mu(\partial^2 P/\partial x^2)$$

$$\partial H/\partial t = cPH - dH^2 + \nu(\partial^2 H/\partial x^2)$$

In the linearised study of small disturbances, one exposes the system to small amplitude perturbations of the initial form

$$P = c_1 \cos qx, H = c_2 \cos qx$$

(Rather general disturbances can be Fourier synthesised from such perturbations.)

The system is seen to have a spatially uniform stable equilibrium

$$P = (ad)/(bc - ed), H = (ac)/(bc - ed)$$

provided $bc - ed > 0$, $c > e$, and

$$R = \nu/\mu < [1/\{\sqrt{(b/d)} - \sqrt{[(b/d) - (e/c)]}\}]^2 = R_{cr}$$

We note that $R_{cr} > 1$.

When $R > R_{cr}$, the local activation effect due to the autocatalytic term interacts with the long range inhibition⁹ to destabilise the uniform state. For R slightly greater than R_{cr} , a perturbation will serve to destabilise if its initial wavelength is approximately equal to $1/q_{cr}$, where

$$q_{cr} = [\sqrt{(b/d)}/\sqrt{[(b/d) - (e/c)]}] - 1$$

By a combination of successive approximations and multiple time scales, we have shown⁵ that for R slightly greater than R_{cr} , the uniform state is replaced by a new steady state in which plant and herbivore are more concentrated in certain regions (with an overall elevation in mean herbivore density). The limit to the growth of the perturbation is a result of the distortion of the initial periodic spatial distribution due to nonlinear interactions.

When two-dimensional effects are introduced, we expect (as happens in the discrete spatial case) that perturbations of large enough amplitude will be shown to destabilise systems which are stable to small perturbations, and that generally the new steady state which arises from instability of the uniform state will either be net-like or spot-like, depending on a certain combination of parameters. This type of analysis is valid for initially homogeneous regions that are large compared with $1/q_{cr}$.

We emphasise that we offer here only one mechanism for generating pattern—not necessarily the principal one. In eventually distinguishing between hypotheses, however, it should be helpful to do the kind of nonlinear analysis we have carried out. To our knowledge this has not previously been done for any of the hypotheses.

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Intercalary regeneration in imaginal wing disk of *Drosophila melanogaster*

IN this paper we show for the first time that the developmental capacity of imaginal disk cells from *Drosophila* can be altered by appropriate grafting operations. The appendages of amphibians and immature arthropods, and the imaginal disks of holometabolous insects, are capable of undergoing pattern regulation when parts are removed. (Pattern regulation is used here to describe the alteration of cell fates in response to an

abnormal situation in a developing system; we include under this term both regeneration and duplication.) Regulation then occurs during growth of the tissue by cell division, a process termed epimorphosis¹. A characteristic feature of epimorphic regulation which has recently been recognised, is the different regulative responses shown by complementary pieces. For example when an imaginal disk of *Drosophila* is bisected and allowed to grow for some time, one of the two fragments usually regenerates the missing parts, while the other undergoes duplication of the presumptive pattern already present². Similarly, whereas the stump of an amputated amphibian or cockroach limb can regenerate distally, the amputated distal part, if kept alive by grafting to a host animal, can also undergo pattern regulation by forming the distal pattern elements, thereby duplicating itself³⁻⁶.

It has been suggested^{1,2} that the complementarity between regeneration and duplication can be understood if it is assumed that new positional values¹ can be generated during growth only in a fixed sequence, a proximal-to-distal sequence in the case of a regenerating limb⁷. The spatial pattern of differentiation thus could be established by a temporal sequence of events occurring autonomously in the dividing cells as the tissue grows out from the cut edge. Under certain circumstances, however, it is clear that the developmental fate of a cell is a function not only of its history but also of its position with respect to other cells. For example, during regeneration from the stump of a cockroach leg or during duplication from the distal leg fragment, the newly produced cells attain positional values only more distal than those of their progenitors. But when the distal part of a leg is grafted to a stump at a more proximal level, intercalary regeneration occurs between the graft and the stump, and the cells produced by the graft can attain more proximal positional values⁸. The results of other graft combinations indicate that the graft can have a similar effect on the development of the stump⁹. Similar results

Fig. 1 Simplified fate map of the imaginal wing disk of *Drosophila melanogaster*, showing only the markers which are genotypically recognisable in our combinations (abbreviations as in Table 1). From Bryant¹³. The positions of the cuts used to generate the fragments are also indicated.

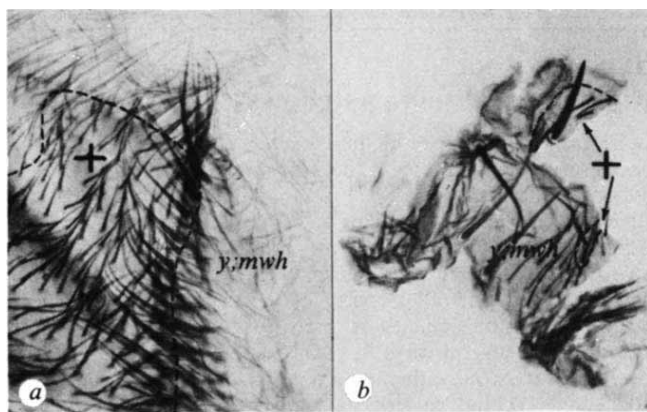
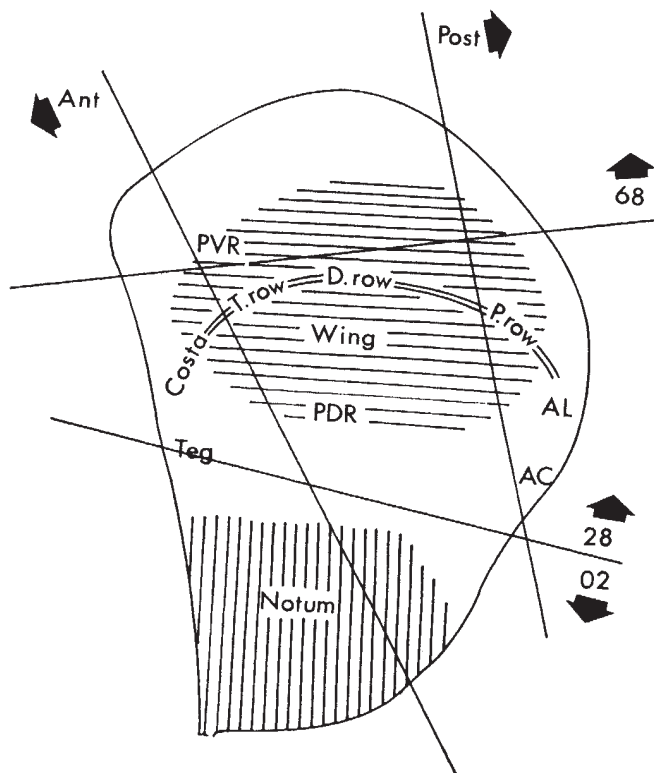


Fig. 2 a, Mosaic wing spread and posterior row produced by a 02/28 combination. The arrangement of posterior row bristles of alternate genotype is similar to that found in mosaics produced *in situ*^{19,20}. b, Mosaic notum produced by a +02/y:mwh 68 combination.

have been reported for the legs of other insects¹⁰, for regenerating amphibian limbs¹¹ and for developing chick limbs¹².

When a fragment of a *Drosophila* imaginal disk is transplanted to a mature host larva, it undergoes metamorphosis with the host, and the cells of the fragment differentiate those structures which they would have formed *in situ*. This has made possible the establishment of detailed fate maps of several of the imaginal disks, and that for the wing disk is shown in Fig. 1. If, however, the fragment is cultured in an adult abdomen for several days (to allow for growth) before transfer to a host larva for metamorphosis, it can either regenerate the missing parts or duplicate the presumptive pattern already present^{2,14}. In the case of the wing disk, it was possible to localise a point in the disk which defines the direction in which regeneration usually occurs; fragments with cut surfaces facing away from this point show regeneration, whereas those with cut surfaces facing in the direction of this point show duplication¹³.

In our first experiments, we studied the interaction of the presumptive notum (02 piece; Fig. 1) with the presumptive wing (28 piece). When cultured alone, the 28 piece frequently regenerates the missing notum whereas the 02 piece undergoes duplication¹³. Individual 02 pieces of one genotype (wild-type *Oregon-R* or *y:mwh*) were mechanically mixed with individual 28 pieces of the other genotype. Mixing was accomplished with two tungsten needles in a drop of buffered Ringer's solution, the two fragments being folded together about 20 times so that they formed a coherent mass. The combinations were transplanted to the abdomens of fertilised female adults, where they remained for 7 d. They were then dissected out of the adult hosts and re-injected into larval hosts for metamorphosis; the larger cultured combinations had to be cut into two or more fragments before they could be transplanted to larval hosts. The differentiated implants were recovered from the metamorphosed hosts and mounted between coverslips for scoring of the structures of each genotype. Control combinations were similarly produced from two 02 or two 28 pieces of different genotypes. The results show that many structures which are never produced by isolated 02 fragments¹³ or 02 combinations (02/02, Table 1) are produced with a high frequency by cells from the 02 piece when in combination with cells from a 28 piece (02/28, Table 1). The 28 piece could simultaneously regenerate the notum, but this is not remarkable since it occurs in cultured isolated 28 pieces¹³ or 28 combinations (28/28, Table 1). Many of the patterns produced by the 02/28 combinations were mosaic, containing cells of both genotypes (Fig. 2a) and patterns were frequently duplicated.

In this experiment all of the positional values regenerated

Table 1 Numbers of cases in which specific structures are formed from combinations of wing disk fragments cultured for 7 d in adult hosts followed by transfer to larvae for metamorphosis

	02/28 <i>n</i> = 29		02/02 Control <i>n</i> = 12	28/28 Control <i>n</i> = 13	02/68 <i>n</i> = 27		68/68 Control <i>n</i> = 10	Ant/Post <i>n</i> = 21		Ant/Ant Control <i>n</i> = 12	Post/Post Control <i>n</i> = 11
	From 02	From 28			From 02	From 68		From Ant	From Post		
Notum	29	24	12	11	27	6		21		12	
Tegula (Teg)	11	17	3	12	14	7		19		12	
Costa	4	21		13	8	9		14		9	
Triple (T.) row	2	26		13	6	7		11		1	
Double (D.) row	8	25		12	7	9	5	15	12		
Posterior (P.) row	11	26		11	10	12	4	6*	15		8
Alar lobe (AL)	6	24		10	6	11	2	1*	13		11
Axillary cord (AC)	6	21		8	3	9	1	1*	6		10
Proximal radius (dorsal) (PDR)	9	22		12	12	1		14			
Proximal radius (ventral) (PVR)	5	16		9	2	10	7	12	1*		
Wing	20	29		13	13	27	10	19	20	1	11

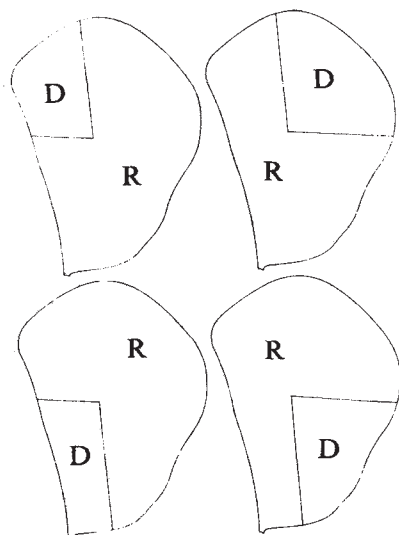
*Regeneration across the anterior-posterior compartment boundary.

y; *mwh* and wild type were used in reciprocal combinations and the results combined. 02, 28, 68, Ant and Post represent the fragments illustrated in Fig. 1. Structures not found in control combinations of identical fragments but appearing in combinations of two different fragments are shown in bold. Combinations producing extensive allotypic structures following transdetermination are not included in these results.

by the 02 piece are already present in the 28 piece with which it is mixed; this suggests that the positional values of the cells derived from the 02 piece are merely being copied from adjacent cells from the 28 piece. In a second experiment, we tested for regeneration of structures which are not normally produced by either fragment, even when cultured. We mixed 02 pieces (presumptive notum) with 68 pieces (presumptive ventral wing blade, ventral hinge, and pleura). Both fragments showed duplication when cultured separately¹³, but in combination they showed intercalary regeneration of many of the structures not normally produced by either of them (02/68, Table 1). Again, the patterns produced were frequently mosaic and often duplicated (Fig. 2*b*). In six cases, cells from the 68 fragment produced parts of the notum, usually derived from the opposite end of the disk (Fig. 1).

We have also tested for intercalary regeneration in the anterior-posterior direction in the wing disk (Table 1). Fragments totally within the anterior compartment¹⁵ were mixed with fragments totally within the posterior compartment (Fig. 1). Each of these fragments is taken from the edge of the disk, and is therefore expected to duplicate¹³; accordingly, the control combinations of anterior and posterior fragments (Ant/Ant and Post/Post, Table 1) produced only the structures expected from the fate map.

Fig. 3 Regenerative behaviour of sectors of the wing disk. In each case, the complementary 90° and 270° sectors were cultured in adult abdomens for 7 d before transfer to host larvae for metamorphosis. R, Regeneration; D, duplication.



When mixed together, however (Ant/Post, Table 1), they showed intercalary regeneration of intermediate structures as in the 02/68 combination, but in most cases each of the fragments in the combination regenerated only those structures within its own (anterior or posterior) compartment, so that the frequency of mosaic patterns was much lower than in the previous experiments. Regeneration of specific structures in the other compartment occurred in nine cases, but this might be an underestimate of the true frequency of regeneration across the compartment border since anterior and posterior wing blade are indistinguishable in implants, and regeneration of one from the other cannot be detected. In our first and second series of experiments the wing-notum, proximal-distal and dorsal-ventral compartment boundaries were clearly transgressed, but in the case of the dorsal-ventral boundary we found some evidence for the maintenance of compartmentalisation since the genotypic boundary in mosaic patterns often coincided with the wing margin bristles or hairs, as reported previously¹⁶ (Fig. 2*a*).

The occurrence of intercalary regeneration provides a simple explanation for the hitherto puzzling behaviour of certain wing disk fragments when cultured alone. The regulatory behaviour of segments of this disk could be accounted for, as described above¹³, on the basis of the direction faced by the straight cut edges. In the same culture conditions, however, each of four 90° sectors of the wing disk duplicates while the complementary 270° sectors regenerate (P.J.B., unpublished, and see Fig. 3); this cannot be accounted for in terms of the predicted regulatory behaviour of the individual cut edges of the sectors. We have also found, however, that in the first few days of culture, the two cut edges of these fragments heal together (unpublished results). If we assume that intercalary regeneration then occurs between the levels of the two fused edges, the results are easily explained. Intercalary regeneration can also account for the ability of imaginal disks to produce a normal pattern after more than half of the cells are killed by X rays (our unpublished results).

Our results also offer a way of understanding pattern reconstruction by dissociated imaginal disks. Mosaic patterns of the kind illustrated in Fig. 2*a* and *b* have been produced in many experiments where genetically marked disks are dissociated into cells and cell clumps, and reaggregated before culture and metamorphosis¹⁶⁻¹⁸. If mosaic patterns can develop on a large scale by intercalary regeneration between fragments from opposite ends of the disk, it seems logical to assume that the same process operating on a smaller scale could account for pattern reconstruction in dissociated and reaggregated disks. This kind of interaction

between cells obviates the need to assume that extensive cell migration¹⁷ is necessary for pattern reconstruction.

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Are morphogenetic tissue interactions mediated by transmissible signal substances or through cell contacts?

TRANSMISSION of morphogenetic signals between embryonic cells is a special form of intercellular communication which may involve diffusible signal substances^{1,2} or intercellular contacts^{3,4}. We have previously presented evidence that the induction of metanephric kidney tubules is dependent on cell contacts between the interacting tissues rather than on diffusible signals^{6,7}. Several embryonic and neoplastic tissues possess an inductive effect when tested against metanephric mesenchyme⁸⁻¹¹. If different inductors release an extracellular, transmissible signal substance, the restriction of induction by interposed filters with varying pore sizes should be independent of the type of inductor tissue, assuming that there is no major difference in size between signal substances. If cell contacts and thus ingrowth of cytoplasmic processes into the filter are required, however, there may be differences between the transfilter inductive capacity of various inductor tissues depending on their ability to send cytoplasmic processes through the filter. That is now shown to be the case, in a comparison of the spinal cord and the mesenchyme of embryonic salivary gland as inducers of metanephric mesenchyme.

Direct combination of salivary mesenchyme with metanephric mesenchyme resulted in the formation of well shaped, coiling tubules with high mitotic activity in all 6 cases, which indicates that salivary mesenchyme like spinal cord is a potent inductor in direct combination. When a Millipore 'TA' filter (pore size 0.8 μm , thickness 25 μm) was interposed between the tissues only 1 of 7 explants showed any tubule response. In the other explants, the metanephric mesenchyme spread out on the filter with good viability but without tubule formation. When spinal cord was used as an inductor, transfilter tubule formation was regularly seen¹⁴.

Nuclepore filters with pore sizes between 0.2 and 0.6 μm allowed the passage of the inductive signal from the spinal cord to the responding metanephric mesenchyme but, in

identical conditions, prevented passage of the signal from the salivary mesenchyme (Table 1). When filters with a nominal pore size of 3.0 μm separated the salivary and kidney mesenchymes, tubules were observed in 8 of 14 explants. These filters, however, allowed the passage of whole metanephric cells through the filter and kidney tubules were often seen below the filter intermingled with salivary cells.

Light microscopy of glutaraldehyde fixed specimens with salivary mesenchyme as inductor showed the presence of cytoplasmic material throughout the 3.0- μm pores, and variable amounts of stainable material in the 0.6- μm pores, and this was confirmed by transmission electron microscopy. This contrasts with the abundant penetration of spinal cord material into even smaller pores⁶.

Scanning electron microscopy of filters where tissues were grown on one side only, demonstrated abundant cytoplasmic penetration through 3.0- μm pores when either salivary mesenchyme or spinal cord was cultured for 48 h (Fig. 1a). Processes from the spinal cord were seen to penetrate even the smallest pores (0.1 μm) whereas salivary mesenchyme never protruded through pores of twice that size (Table 1). Four out of seven explants with salivary mesenchyme cultured for 48 h on the lower surface of a filter with 0.6 μm pores showed penetration of cytoplasmic processes in restricted areas. In contrast, there were abundant processes when spinal cord was cultured in identical conditions (Fig. 1b). When metanephric mesenchyme was cultured on top of various Nuclepore filters, cell processes (and whole cells) penetrated to the lower surface only through the largest pores.

Thus the two inductors, spinal cord and salivary mesenchyme, gave identical induction responses in direct combination with the responding metanephric mesenchyme, but showed marked differences in inducing capacity when separated from the responder by a filter. While spinal cord was occasionally able to pass an inductive signal through 0.1 μm pores and did so regularly through larger channels, salivary mesenchyme required pores ten times as large to send the morphogenetic signal to the responding mesenchyme. If the message is carried by transmissible molecules, the results imply that the signal molecules are markedly different in the two inductor tissues, and that the 'salivary factor' is trapped by $\sim 0.6\text{-}\mu\text{m}$ pores. Measurements of the passage of various molecules through Nuclepore filters have not lent support to such an idea, as compounds with a molecular weight as high as 10^7 easily pass through even the smallest pores (0.1 μm) (ref. 6). It seems more reasonable to correlate tubule induction to the differences in the penetration of cytoplasmic material through the filters. Spinal cord is able to send thin filamentous processes through 0.1- μm pores, whereas salivary mesenchyme requires a minimum

Table 1 Tubule-forming response of metanephric mesenchyme and penetration of cytoplasmic processes from the inductor as a function of the type of the inductor tissue and the pore size of the interposed Nuclepore filter*

Filter Nominal pore size (μm)	Inductor			
	Salivary mesenchyme Tubules	Penetration	Spinal cord Tubules†	Penetration
0.1	‡	—	3/30	+
0.2	0/13	—	27/31	++
0.5-0.6	0/10	±	25/25	++
3.0	8/14	+	15/15	++

*Metanephric mesenchyme and spinal cords were dissected from 11-day mouse embryos (CBA/T6T6 $\times$ BALB/c), and salivary mesenchymes from the submandibular gland of 13-d embryos. Tubule formation was evaluated by light microscopy after 72 h of cultivation¹². Nuclepore filters with a thickness of $\sim 15\text{ }\mu\text{m}$ and a nominal pore size of 0.2, 0.6 or 3.0 μm (General Electric Co., Pleasanton, California, USA) were used. According to previous measurements, the largest pores are, however, 1.5-2.0 μm in diameter⁶.

‡Results from ref. 6.

†Not determined.

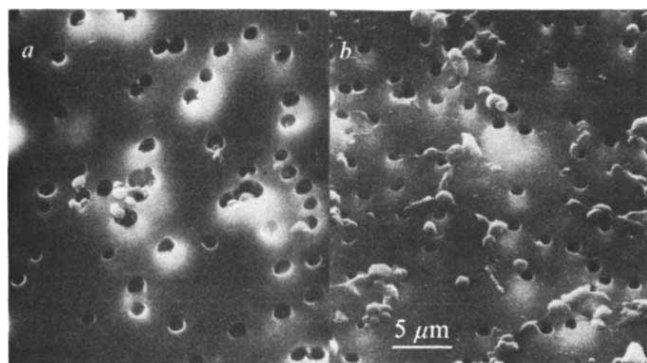


Fig. 1 *a*, Scanning electron micrograph of the upper surface of a Nucleopore filter with a nominal pore size of 3.0 µm. Salivary mesenchyme cultured for 48 h on the lower surface of the filter can be seen to send cytoplasmic processes through the pores. *b*, Scanning electron micrograph of the upper surface of a 0.6-µm pore size Nucleopore filter with a piece of spinal cord cultured on the lower surface for 48 h. Numerous cytoplasmic processes can be noted on the upper surface of the filter membrane. Tissues were placed on one side of the filter only. The inductor tissues (spinal cord, salivary mesenchyme) were placed below the filter and the responding metanephric mesenchyme on top of it. After 48 h of cultivation, the explants were rinsed in PBS, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.8 for 60 min at room temperature, dehydrated in ethanol and dried by the critical point method¹³. Subsequently the specimens were covered with a thin layer of carbon and gold in a Balzers Micro-BA-3 evaporator and examined in a Jeol JSM-U3 scanning electron microscope (the Electron Microscope Laboratory, University of Helsinki).

pore size of 0.6 µm. Furthermore, induction of kidney tubules was never seen in transfilter situations which did not allow cytoplasmic penetration and development of contacts between inductor cells and the responding mesenchyme. The results also show that cytoplasmic penetration of filters can occur without the passage of the morphogenetic signal. The reason for this may be that contacts are either established too late, as the penetration time is a function of pore size⁶, or are too few. In another interactive system where the response can be quantified, the intensity of induction is a function of pore size and filter thickness¹⁵, and may correlate to the size of the contact area.

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Haploid mouse blastocysts developed from bisected zygotes

INTEREST in haploid mouse embryos as a material for studying gene action in early development and as a potential source of haploid cell lines has stimulated a number of attempts to produce this condition experimentally. We describe here a new method of producing haploid mouse embryos by bisecting fertilised eggs. Morphologically normal haploid and haplo-diploid mosaic blastocysts were formed after insertion of such fragments to empty zonae and transfer to the oviduct.

Haploid blastocysts can now be routinely obtained after parthenogenetic activation of unfertilised eggs (for review, see refs 1–3). When transferred to ectopic sites such embryos give rise in a small proportion of cases to 'growths' composed of many types of differentiated tissues, some of which may be haploid^{4,5}. No success has yet been reported, however, in establishing haploid mammalian cell lines in culture. Haploid mouse embryos can also be obtained after microsurgical removal of one pronucleus from the fertilised egg, but for unknown reasons, very few develop into blastocysts⁶. Another possible method of producing haploid embryos from fertilised eggs would be to cut the pronucleate egg into halves, each containing one pronucleus. A.K.T. has recently developed such a technique (unpublished).

Pilot experiments showed, however, that early one-cell eggs subjected to harsh manipulation including microsurgery do not develop very well in culture and we decided, therefore, to test the viability of the bisected eggs in optimal conditions—that is, *in vivo*—before attempting a large scale experiment *in vitro*. As the zona pellucida is removed from the egg during the cutting procedure, the egg fragment must be inserted back into empty zonae before transfer to recipients because, up to the eight-cell stage, zona-free eggs do not survive in the oviduct^{7,8}.

Spontaneously ovulating F₁(CBA × C57BL/10) females mated with CBA-T6T6 males, and 129/J females mated with 129/J males, were killed in the early afternoon (1400–1530) on the day of the vaginal plug. The eggs were treated with hyaluronidase in phosphate-buffered saline (PBS) (200 IU ml⁻¹) to remove cumulus cells, subjected to 0.5% Pronase to dissolve the zona pellucida⁹, cut into halves (A.K.T., unpublished) and cultured overnight in Whitten's medium¹⁰ in liquid paraffin. Those in which both halves had divided once were transferred to PBS+0.1% bovine serum albumin and inserted microsurgically into empty zonae prepared by sucking out the contents of unfertilised eggs^{11,12}. Blastomeres in zonae (Fig. 1a) were transferred to the oviducts of mice on day 1 of pseudopregnancy¹³. Three pairs of blastomeres originating from halves containing both pronuclei were also inserted into zonae and transferred to one recipient. Seventy-two hours later (day 4 of pseudopregnancy, day 5 of development of eggs) the genital tracts were flushed with PBS and the recovered embryos cultured for 3–4.5 h in medium containing Colcemid (0.2 µg ml⁻¹) and examined in air-dried preparations¹⁴.

Table 1 summarises the results. The overall effectiveness of transplanting pairs of blastomeres into empty zonae was about 65% (range 50–100%). The recovery of transplanted eggs was, however, very low—only 16.6%. The collected embryos included six blastocysts (9%), two morulae, and three irregular embryos arrested in cleavage. These embryos together with empty zonae found in some recipients accounted for 40.3% of transplanted eggs. The majority of transplanted pairs of blastomeres most probably escaped from the zonae through the incision shortly after being transferred to the oviduct, and degenerated¹¹.

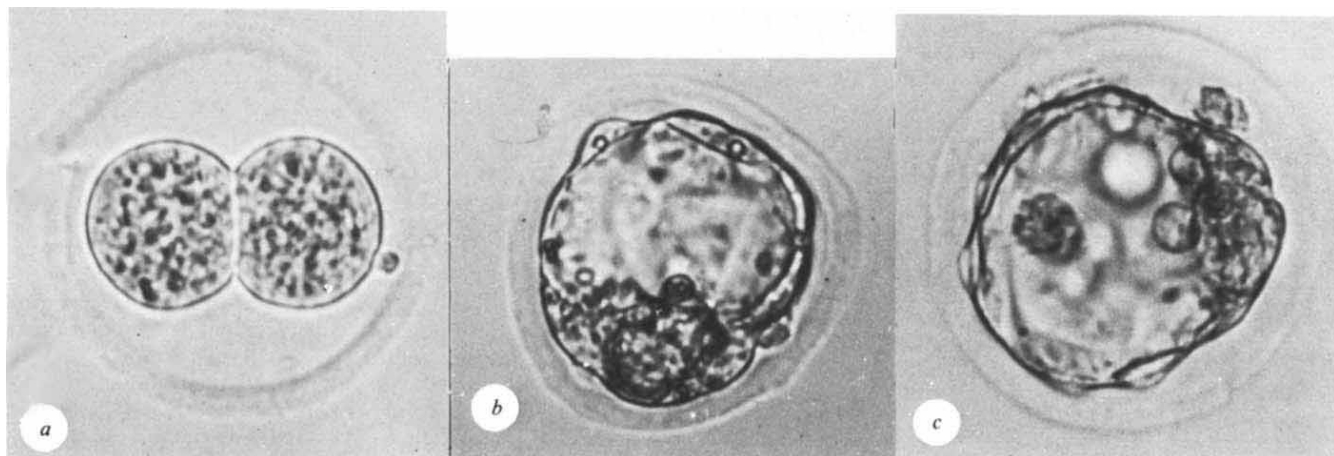


Fig. 1 *a*, Pair of blastomeres developed *in vitro* from a haploid egg half and transplanted into empty zona pellucida ($\times 450$). *b*, 4.5-d-old gynogenetic haploid blastocyst of $F_1(\text{CBA} \times \text{C57BL}/10)$ genotype ($\times 600$). *c*, 4.5-d-old diploid blastocyst of $F_1(\text{CBA} \times \text{C57BL}/10)$ genotype developed from an egg half which contained both pronuclei ($\times 450$).

Other factors which may have been responsible for the low recovery are degeneration due to injury produced by the two successive microsurgical operations and possibly also early death of androgenetic haploids lacking an X chromosome (diploid OY embryos are believed to die during early cleavage¹³). The last factor could account for the loss of 25% of transplanted haploids.

In spite of the low effectiveness, these experiments show that haploid egg fragments obtained by bisecting the pronucleate egg are viable and can develop into regular blastocysts composed of both trophoblast and inner cell mass (Fig. 1*b*). The number of cells (nuclei+metaphase plates) in the six available blastocysts were as follows: 29, 32, 38, 39, 57, and 68. In three blastocysts all metaphase plates were haploid but the other three had both haploid and diploid plates ($4 \times n + 3 \times 2n$; $2 \times n + 1 \times 2n$; $8 \times n + 1 \times 2n$) thus showing that diploidisation of some cells during cleavage which leads to $n/2n$ mosaicism, is not peculiar to parthenogenetic haploids^{16,17}.

In the case of $F_1(\text{CBA} \times \text{C57BL}/10)$ eggs fertilised by CBA-T6T6 sperms it was possible to determine the gynogenetic or androgenetic origin of embryos on the basis of the presence or absence of the T6 marker chromosome. Out of five such blastocysts four definitely had no T6 chromosome and were therefore gynogenetic. In the fifth blastocyst the presence of the marker chromosome was suspected, but because the quality of the only complete metaphase plate was not very good, the ability of androgenetic halves to develop to blastocysts remains to be confirmed. The 129/J blastocyst clearly did not contain a Y chromosome (Fig. 2) but because of the lack of karyological

markers it was not possible to determine whether it carried the genome derived from the egg or from the sperm.

The only embryo recovered after transferring three pairs of blastomeres derived from egg halves containing two pronuclei, was a regular blastocyst (Fig. 1*c*) composed of 42 cells and containing in the karyotype—according to expectations—one T6 chromosome.

In view of these encouraging results attempts have been undertaken to analyse in detail development of haploid egg

Fig. 2 Haploid metaphase plate from a blastocyst developed from a bisected 129/J egg. There is no Y chromosome in the karyotype but because of the lack of chromosome markers the origin of the blastocyst (gynogenetic or androgenetic) cannot be determined.

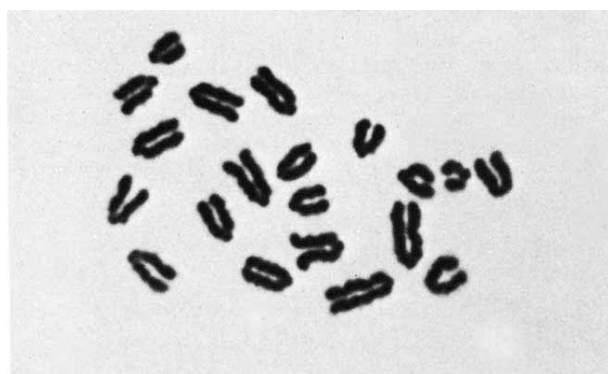


Table 1 Effectiveness of transplanting pairs of blastomeres into empty zonae

Ploidy	Genotype of eggs	No. of cleaved halves used for insertion into empty zonae	No. of cleaved halves successfully inserted and transferred to recipients	No. of transfers	Stage and no. of recovered embryos (+empty zonae)
n	$\text{♀ } F_1 (\text{CBA} \times \text{C57BL}/10)$ $\times$ ♂ CBA-T6T6	93	60	11	5 Blastocysts 2 Morulae 2 Irregular embryos arrested in cleavage 13 Empty zonae
	129/J $\times$ 129/J	9	7	2	1 Blastocyst 1 Irregular embryo arrested in cleavage 3 Empty zonae
$2n$	$\text{♀ } F_1 (\text{CBA} \times \text{C57BL}/10)$ $\times$ ♂ CBA-T6T6	3	3	1	1 Blastocyst

fragments *in vitro* in conditions which enable the fate of sister halves from each egg to be followed.

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Somatic cell genetic evidence for X-chromosome linkage of three enzymes in the mouse

THE X linkage of loci coding for glucose-6-phosphate dehydrogenase (G6PD), hypoxanthine-phosphoribosyl transferase (HPRT), and phosphoglycerate kinase (PGK) has been determined for the mouse species *Mus musculus* and *M. caroli*. Evidence was obtained from a somatic cell genetic analysis of cultured embryonic cells of an interspecific hybrid foetus. The X-chromosomal assignment of

these loci has been determined already for several other mammalian species. This evidence was obtained from genetic analyses of electrophoretic variants¹ and enzyme deficiencies^{2,3}; from the expression of these genes in viable interspecific hybrids⁴⁻⁶; from somatic cell genetic analyses of heterozygotes⁷⁻⁹, and from the expression of these loci in interspecific somatic cell hybrids¹⁰.

Evidence that these loci are X-linked in the laboratory mouse, *M. musculus*, comes from the observation that both X chromosomes are active in female germ cells and that gene dosage for these loci is doubled in oocytes of normal XX females as opposed to XO females. Activity measurements of G6PD¹¹, HPRT¹², and PGK¹³ in oocytes has shown that oocytes from XX females contain about double the activity of each of these enzymes compared with oocytes from XO females. No genetic variants for any of these enzymes have been reported in the mouse to establish independently X linkage of these loci.

We have, however, observed that *M. musculus* and *M. caroli*, a related species from Dr J. T. Marshall in Thailand, have different electrophoretic mobilities for a number of enzymes including G6PD, HPRT and PGK. Hybrids of *M. caroli* and *M. musculus* have not been reported in the wild and they cannot be mated in laboratory conditions. Both species, however, have 40 telocentric chromosomes¹⁴ and a similar reproductive cycle (V.M.C., unpublished). Interspecific hybridisation between *M. caroli* and *M. musculus* was attempted using artificial insemination of *M. musculus* females with *M. caroli* sperm (unpublished results of V.M.C. and J. Karr). No hybrid offspring were born but successful implantation and development to the foetus stage (approximately 14-16 d) was observed in three cases.

A critical feature of X-gene expression is that gene dosage is doubled in females compared with males, but the levels of gene products between the sexes is equal. The basis of this compensation between the sexes is attributable to the inactivation of one of the X chromosomes in all the somatic cells of the female^{15,16}. This inactivation occurs early in mammalian development, possibly by the blastocyst stage. Maternally and paternally derived X chromosomes are inactivated at random so that the adult female has

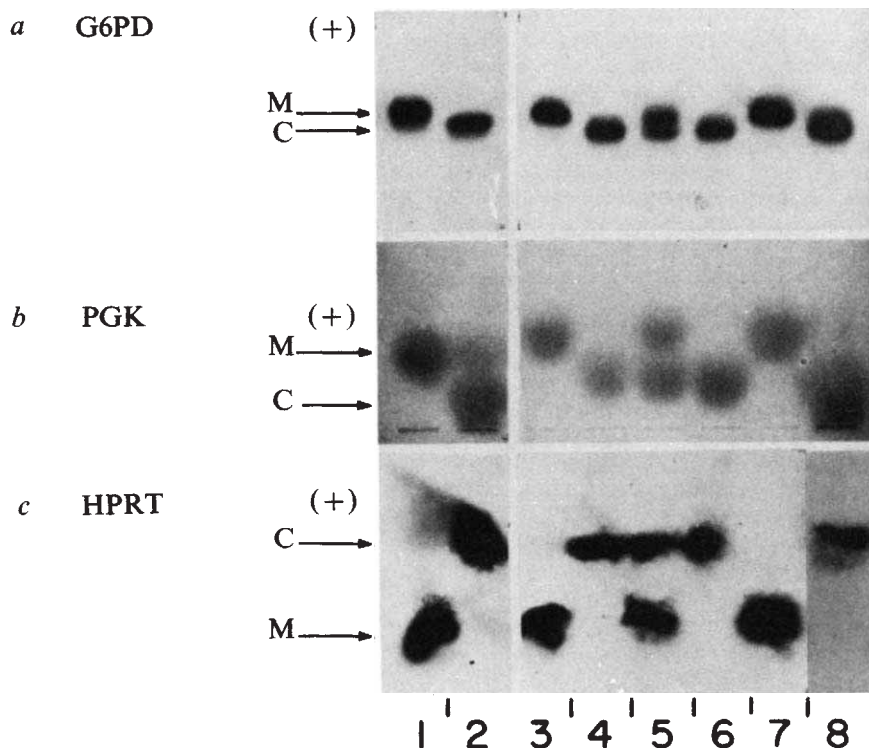


Fig. 1 Electrophoretic patterns of G6PD (a), PGK (b) and HPRT (c) in tissue and tissue culture homogenates. M represents the *musculus* forms and C represents the *caroli* forms. 1, *musculus* kidney; 2, *caroli* kidney; 3, clone B-5; 4, clone B-1; 5, cell population C; 6, clone C-6; 7, clone C-4; 8, clone A-1. The prefix for each clone indicates the mass population from which the clone was derived. Homogenates of cells from cell population A and B and mixtures of kidney homogenates demonstrated the same phenotype as cell population C in channel 5. The apparent extra PGK band in *caroli* kidney (b, 2) is a minor anodal band in the PGK phenotype that is present at high concentrations of tissue extracts. These minor components were observed in tissues of both males and females of *M. caroli*. A similar minor component is seen in tissue extracts of *M. musculus*, but at even higher concentrations. These minor components were not observed in cell culture extracts.

Table 1 Distribution of gene markers in clones of cultured cells derived from a *M. musculus* × *M. caroli* foetus

Population	Total clones	Six linked enzymes			Autosomal enzymes		
		G6PD	PGK	HPRT	NP	GPI	GOT-1
A	1	MC	MC	MC	H	H	H
		C	C	C	H	H	H
B	2	MC	MC	MC	H	H	H
		C	C	C	H	H	H
C	5	M	M	M	H	H	H
	5	MC	MC	MC	H	H	H
		C	C	C	H	H	H
		M	M	M	H	H	H
	3						
	16						

For each clone, all enzymes were analysed on the same cell homogenate. M denotes *musculus* origin; C denotes *caroli* origin; MC denotes both *musculus* and *caroli* contributions; and H denotes a hybrid phenotype with parental and heteropolymeric electrophoretic forms.

two populations of cells; one expressing the maternal and the other the paternal X.

If G6PD, HPRT and PGK are X-linked in *Mus* species, we reasoned that both *musculus* and *caroli* forms would be expressed in a hybrid female foetus and only the *musculus* form would be expressed in a male foetus. We therefore expected that a primary cell culture of hybrid cells would have both *caroli* and *musculus* electrophoretic forms if the foetus were female, or only *musculus* forms in the case of a male. The resulting cell lines from the hybrid foetus had both *caroli* and *musculus* electrophoretic forms of G6PD, HPRT and PGK and were presumed to be female. In the case of a female we further reasoned, from the Lyon hypothesis, that clones derived from single cells of the culture should uniformly express either the *caroli* or *musculus* electrophoretic forms of all three enzymes if these loci are X-linked. Hybrid foetal electrophoretic phenotypes and cloning of cultured foetal cells showed that these enzymes segregated as predicted for X-linked loci in *M. caroli* and *M. musculus*.

Foetuses were dissected from the uterus 16 d after arti-

cial insemination. Two were homogenised in equal volumes of distilled water and assayed for electrophoretic phenotypes. The third foetus was used to establish a primary cell culture: the 16-d embryo was minced in a solution of proteolytic enzymes (Viocase, Gibco) and Eagle's medium without serum, and divided into three parts, each of which were plated separately in Dulbecco's modified Eagle's medium (DMEM) (high glucose formula) with antibodies and 10% foetal calf serum (Gibco). The cells were maintained in an atmosphere of 5–7% CO₂. A monolayer of proliferating cells was obtained from each of the three original cell suspensions and designated primary cell populations A, B and C. Each cell population was maintained separately in the above medium and single cells were cloned by the procedure of Puck *et al.*¹⁷. Cell populations and clones were grown to confluency, collected for enzyme identification, and homogenised at a concentration of 7 × 10⁷ cells per ml 0.5 M Tris at pH 7.5 (ref. 18). G6PD (EC 1.1.1.49) PGK (EC 2.7.2.3) and HPRT (EC 2.4.2.8) were analysed by starch gel electrophoresis^{10,19}.

Electrophoretic phenotypes of *M. caroli* and *M. musculus*

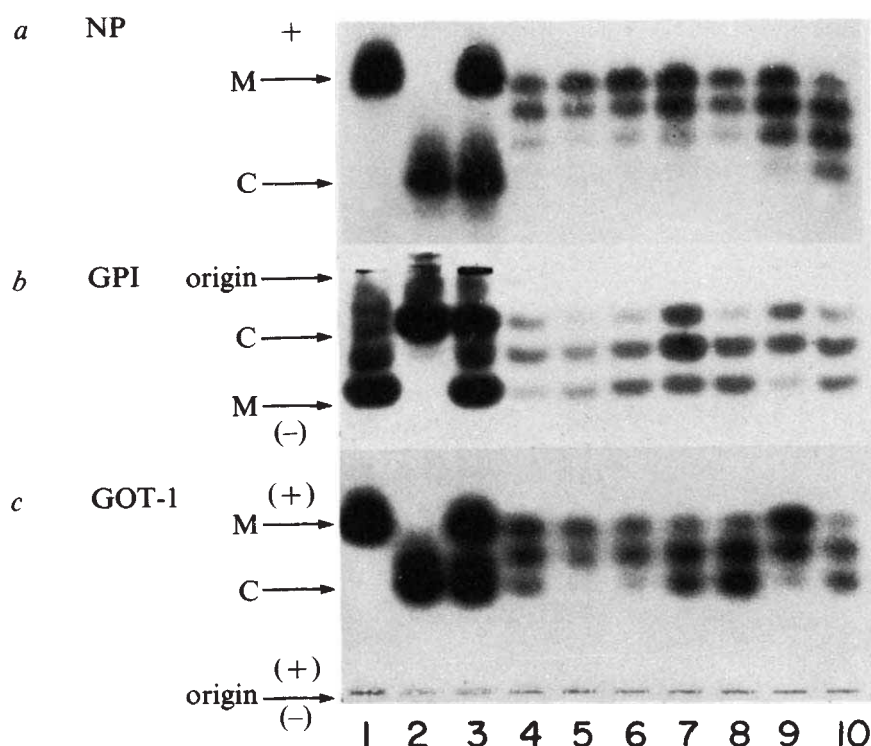


Fig. 2 Electrophoretic patterns of: NP (a), GPI (b) and GOT-1 (c) in tissue and tissue culture homogenates. M represents *musculus* forms and C represents *caroli* forms. 1, *musculus* kidney; 2, *caroli* kidney; 3, 1:1 mixture of *musculus* and *caroli* homogenates; 4, cell population B; 5, clone B-5; 6, clone B-1; 7, cell population C; 8, clone C-6; 9, clone C-4; 10, clone A-1. In cell culture extracts, the two heteropolymers migrating between the parental forms of NP indicate that NP is a trimeric enzyme²¹. The single heteropolymer observed for GPI and GOT-1 in cell culture extracts indicates that these enzymes are dimers. All cell culture extracts of hybrid populations demonstrate both parental and heteropolymer forms, indicating that both parental genes for each enzyme are active in each cell. The *musculus* and *caroli* tissue GPI phenotypes possess a major band and several anodally migrating minor bands at the high concentrations used for tissue extracts (b, 1 and 2). These minor bands were not observed in cell culture extracts. GPI is a dimeric enzyme and a hybrid enzyme is observed in heterozygotes for GPI electrophoretic variants in the mouse²². The band intermediate to the two parental forms in the 1:1 mixture (b, 3) is a *musculus* minor component and not a heteropolymer. A hybrid GPI enzyme, compared with the parental forms, is more intense as demonstrated by the relative intensities of the parental and hybrid enzymes in the cell culture samples. The variability between clones with regard to intensity of enzyme bands probably represents an increase in the number of

gene copies since chromosome numbers increased as a result of the foetal cells becoming established as cell culture lines. Chromosomes were examined on two hybrid clones from cell population B, one expressing *musculus* and the other *caroli* X-linked enzymes. They had mean chromosome numbers of 69 and 63, respectively, and only telocentric chromosomes were observed.

kidney HPRT, PGK and G6PD are shown in Fig. 1. The major band of activity for each enzyme has a different mobility for each species. One hybrid foetus demonstrated both *musculus* and *caroli* forms and was presumably a female, and one foetus demonstrated only *musculus* forms and was presumably male. These findings are consistent with X linkage. In each of the three primary cell populations, the electrophoretic patterns consisted of both *musculus* and *caroli* forms for each enzyme. The electrophoretic phenotypes of the three enzymes for cell population B is shown in Fig. 1 (channel 5). Sixteen clones derived from single cells were examined. The clones were established from all three populations soon after the establishment of the primary cell culture. Eight of the clones coordinately expressed only *musculus* electrophoretic types for PGK, HPRT and G6PD and eight coordinately expressed only *caroli* forms (Fig. 1 and Table 1). These findings support the prediction that the primary cell populations are mixtures of cells with either an active *musculus* X chromosome or an active *caroli* X chromosome.

The expression of three autosomal enzymes which differ in electrophoretic phenotype between the two species was tested to demonstrate that the foetuses, the cells of the primary cell populations and the clones were true hybrids with functional *musculus* and *caroli* genes in the same cell. Cytoplasmic glutamic oxaloacetic transaminase (GOT-1) (ref. 20), nucleoside phosphorylase (NP) (ref. 21), and glucosephosphate isomerase (GPI) (ref. 22) are multimeric enzymes which differ electrophoretically between *musculus* and *caroli* (Fig. 2). Each of these enzymes is coded by an autosomal locus as determined by genetic tests in *M. musculus* or man. Since these enzymes are multimeric, it was expected that electrophoretic phenotypes demonstrating both parental forms and intermediate hybrid forms would be observed if both parental genes were expressed in the same cell. Hybrid electrophoretic patterns consistent with the formation of intermediate heteropolymers by random combination of *musculus* and *caroli* polypeptide subunits in individual cells were demonstrated in the foetuses, the three cell populations and the clones (Fig. 2 and Table 1). Mixtures of *musculus* and *caroli* kidney extracts produced additive electrophoretic patterns without intermediate hybrid enzymes (Fig. 2). All 16 clones demonstrated both parental and heteropolymer bands for the autosomal enzymes (Table 1). Thus, both *caroli* and *musculus* genes were active in the original cell which formed the clones and remained active through successive cell generations. On the other hand, each clone showed only the *caroli* or the *musculus* phenotype for PGK, G6PD and HPRT. We conclude that these results are consistent with the predictions of the Lyon hypothesis that there are two populations of cells, that only one X chromosome is active in a cell, and that the same X remains active in successive cell generations. In retrospect, two populations of cells were indicated in one foetus and in the cells of the hybrid foetus used to make the primary culture by the two banded G6PD electrophoretic pattern. Because G6PD is a dimer, one expects a three banded electrophoretic phenotype like GPI or GOT-1 (Fig. 2) if both chromosomes are active in a single cell. Only both parental forms were observed, however, (Fig. 1). The electrophoretic findings of hybrid enzymes for NP, GPI and GOT-1 demonstrate that both *musculus* and *caroli* genes function in the same cell and that the foetuses were interspecific hybrids.

The strategy of analysing clones from single cells of heterozygous females has demonstrated the X linkage of HPRT, G6PD and PGK in man⁷⁻⁹ and G6PD in mules². In each instance only one of the two possible parental forms demonstrated by a heterozygous female was expressed. Such somatic cell genetic analyses have been confirmed in man by family studies using genetic variants of each enzyme. The segregation of PGK, G6PD and

HPRT in clones of somatic cells from an interspecific hybrid foetus and foetal extract phenotypes demonstrates an X-chromosome assignment of these enzyme loci in mice as reported from a comparison of enzyme activities in XO and XX oocytes¹¹⁻¹³. These conclusions also agree with Ohno's hypothesis of conservation of the X chromosome among mammalian species²⁴.

The relative amounts of *musculus* and *caroli* PGK, G6PD and HPRT expressed in the three primary cell populations were approximately equal. This suggests that the proportion of the cells with active *musculus* or *caroli* X chromosomes may have been equal in the hybrid foetus. Thus, there is no evidence that either parental X chromosome was preferentially inactivated during development or that cell selection acts to distort the proportions of the two cell populations. This finding is similar to the observation of X-chromosome expression in interspecific hybrids such as the mule^{4,5} and in crosses of European hare species⁶, indicating that the genetic mechanism for X-chromosome inactivation is also conserved in the speciation of eutherian mammals.

In conclusion, although we have not yet obtained hybrid progeny developing to term, the combination of (1) multiple X-linked biochemical differences, (2) random inactivation of *caroli* and *musculus* X chromosomes in hybrid embryos, and (3) the occurrence of some hybrid foetuses which seem grossly normal at 14-16 d of development, suggests that the hybrid combination of *M. musculus* and *M. caroli* is a promising experimental system for studying X inactivation in mammalian development.

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Imperfect complementation in human-hamster somatic cell hybrids

SOMATIC cell hybridisation can reveal complementation between genetic markers¹, and interspecific hybridisation provides a means to study the mechanisms involved in

complementation. When the phenotypes of both parental cells are distinguishable at the molecular level, two complementation mechanisms can be recognised². When the allele contributed by the wild type parental cell overcomes the block in the biochemical pathway characteristic of the mutant parent, products characteristic of both species should be present in the hybrid. Alternatively, if either allele acts exclusively on its own pathway, that is the wild-type allele cannot act in *trans*, the blockage due to the mutation remains and only the wild-type product would be expected to be synthesised in the hybrid.

We have reported² complementation of a defect in the production of ribosomal RNA (rRNA) in interspecific cell hybrids. Mutant *ts422E* is a temperature sensitive (*ts*) variant of the Syrian hamster line BHK 21/13 (ref. 3). At the non-permissive temperature (39 °C) the cleavage of nucleolar 32S RNA to produce mature ribosomal 28S RNA is blocked. rRNA processing and production are otherwise normal at 33 °C^{3,4}. When *ts422E* is hybridised with mouse cells the resulting hybrids produce both hamster and mouse 28S RNA at the non-permissive temperature², indicating that in this case complementation occurs by the first of the two possible mechanisms. In an attempt to characterise further the nature of the complementation between interspecific rRNA maturation genes, we hybridised *ts422E* with human cells. We found that complementation of the defect borne by the *ts422E* hamster cell mutant is only partially achieved in hybrids with human cells. In this case the same mechanism of complementation operates as in *ts422E*-mouse hybrids, but only to a partial extent.

ts422E cells were fused with LW SV40-transformed human cells deficient in hypoxanthine phosphoribosyltransferase⁵ in the presence of β -propiolactone-inactivated Sendai virus at pH 8.0 (ref. 6). Hybrid cells were selected in HAT medium⁷ at 39 °C. Hybrid colonies were obtained at a frequency of about 1×10^{-5} of the total cells plated in selective conditions; they were isolated and propagated in HAT medium at 39 °C for one passage. Hybrid lines were propagated subsequently in normal medium at 39 °C unless otherwise stated.

We used populations of hybrids obtained on two plates. The populations grew poorly at 39 °C, but in an essentially

Fig. 1 Hybrid population F3 growing at 39 °C was labelled for 13 h with ³H-uridine (6 μ Ci, 2 μ g ml⁻¹). Cells were washed, the cytoplasmic fraction prepared and the 28S rRNA isolated after centrifugation in sucrose gradients as described before². The RNAs were analysed by polyacrylamide gel electrophoresis. Electrophoreses were run for 24 h at 5 mA per gel. Gels were sliced and counted as described in ref. 2. Samples were run with ¹⁴C-labelled markers prepared from BHK or HeLa cells. *a*: ●, 28S ³H-RNA from population F3; ○, HeLa ¹⁴C-28S RNA. *b*: ●, 28S ³H-RNA from population F3; ○, BHK ¹⁴C-28S RNA.

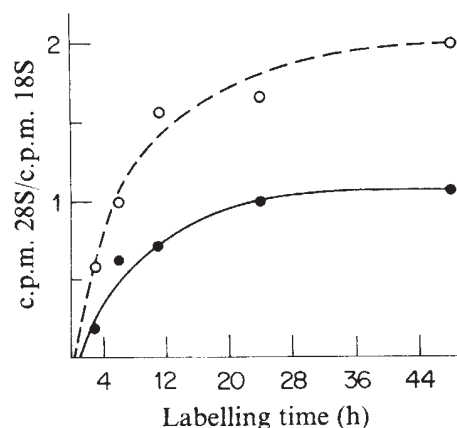
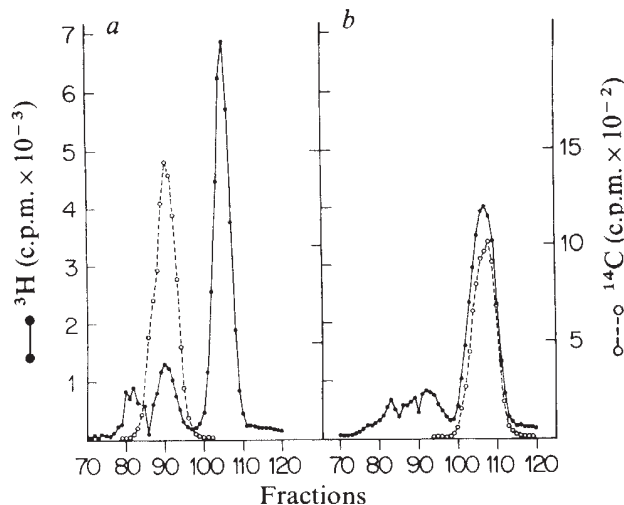


Fig. 2 Plates containing hybrid 53-99 C6 were incubated for 5 d at 33 or 39 °C and labelled for different times with ³H-uridine (16, 8 or 4 μ Ci; 2, 4, or 8 μ g ml⁻¹). At the end of the pulse cells were fractionated as described in caption for Fig. 1. RNA from cytoplasmic fractions was purified by double phenol extraction and precipitated with 2 volumes of 95% ethanol at -20 °C. RNA was subjected to polyacrylamide gel electrophoresis in the conditions described in Fig. 1. Electrophoreses were run for 4.5 h. 28S and 18S rRNA peaks were integrated in each case and the ratio of c.p.m. in the 28S peak relative to the amount of radioactivity in the 18S peak (28S:18S ratio) was determined. ●, Data corresponding to 39 °C; ○, data corresponding to 33 °C.

normal manner at 33 °C. Giemsa banding by a modification of Seabright's method^{8,9} showed that the hybrid F3 contained human chromosomes 3, 14, 15, 17 and 20 and two abnormal chromosomes, probably of human origin. The hybrid F31 had human chromosome 3 and one abnormal human chromosome. It is also expressed human mannose phosphate isomerase (MPI). All hybrid cells contained the entire complement of hamster chromosomes.

The 28S rRNA made by the populations F3 and F31 at 39 °C was analysed in 2.6% polyacrylamide gels, where hamster 28S RNA migrates faster than human 28S RNA¹⁰, as described in Fig. 1. F3 synthesised mostly hamster 28S rRNA but some human rRNA was also present; results were similar for population F31 (Fig. 1).

For subsequent studies we used a clone of the F3 population. The clone 53-99 C6 also grew poorly at 39 °C. It contained human chromosome 3 in five and human chromosome 17 in two of 20 metaphases analysed. These hybrid cells also expressed human MPI activity, but not human pyruvate kinase 3 or hexosaminidase A, suggesting that they contained only a portion of human chromosome 15 (refs 11 and 12). Table 1 compares the doubling times of the 53-99 C6 hybrid at 33 and 39 °C with those of wild-type BHK, the hamster parent *ts422E* and one of the mouse-*ts422E* hybrids, hyC7. Growth in all *ts*⁺ cells was faster at 39 than at 33 °C, with the exception of the *ts422E*-human hybrid, suggesting inefficient, although positive, complementation.

To determine whether the slower growth at 39 °C was a consequence of reduced production of 28S rRNA, the kinetics of labelling of rRNA were studied at this temperature. The amount of radioactivity in 18S rRNA was used as a reference as 18S rRNA is normally synthesised in the parental *ts* mutant at either temperature³. 53-99 C6 cells were labelled for different times, and cytoplasmic RNA was isolated as before and analysed in polyacrylamide gels³ (Fig. 2). At 33 °C the ratio of label in 28S RNA to that in 18S RNA increased, to reach a steady state at ~1.9 after 24 h. At 39 °C the steady state was reached more slowly and the ratio never exceeded 1.2. Similar results were obtained with the original F3 and F31 hybrid populations. Thus, the slow growth of the hybrid at 39 °C was probably due to impaired production of 28S rRNA.

At either temperature, hybrid 53-99 C6 synthesised 28S rRNA of hamster origin only (Fig. 3; results obtained at

Table 1 Doubling times of several cell lines at 33 and 39 °C (h)

	33 °C	39 °C
53-99 C6 (<i>ts422E</i> -human hybrid)	29	37
C7 (<i>ts422E</i> -mouse hybrid)	23	18
<i>ts422E</i>	18	∞
wild-type BHK21	16	12

Plates (60 mm) were seeded with 5×10^4 cells per plate and incubated at 33 or 39 °C. Every 24 h duplicate plates from each temperature were trypsinised and the cells counted with a Coulter counter.

39 °C were identical). This suggests that the human nucleolar organisers^{13,14} have been segregated out in this hybrid, although alternative hypotheses cannot be ruled out.

To determine whether the reduced production of 28S RNA in the hybrids at 39 °C was the expression of the defect of the *ts422E* parent, corrected only to some extent, we analysed the pattern of nucleolar RNA processing. *ts422E* cannot produce 28S RNA because of a block in the processing of the 32S RNA precursor, which, after production is mostly degraded, although a portion of it accumulates to levels 8–10 times greater than in wild-type cells⁴.

Table 2 summarises the distribution of label in nucleolar 32S RNA and cytoplasmic 28S rRNA in hybrid 53-99 C6 after an 11-h pulse at 33 or 39 °C. Data for BHK and *ts422E* at 39 °C are also included. As 32S (28S) and 18S RNAs are derived in a 1:1 molar ratio from a common 45S RNA precursor^{13,14}, the 32S:18S or 28S:18S ratios enable us to normalise the differences due to causes other than the mutation itself, such as differences in uridine pools or uptake in different cell lines. At 39 °C the 28S:18S ratio in the hybrid is intermediate between the values for wild-type and mutant cells; the same was true for the 32S:18S ratio. The 32S:18S ratio showed clearly that much more 32S RNA accumulated in the hybrid at 39 °C than in wild-type BHK, the degree of accumulation being similar to that in *ts422E* cells. The accumulation of 32S RNA in the hybrid, however, was greater than expected at 33 °C, perhaps because the steady state had not yet been reached (Fig. 2). Thus rRNA precursors are processed in *ts422E*-human hybrids at 39 °C in a pattern similar to that in the *ts422E* mutant, with comparable accumulation (and degradation) of the 32S RNA precursor. This indicates that the slow growth of the hybrids at 39 °C is due to imperfect complementation (albeit compatible with survival) of the *ts422E* defect.

Although we wished to map the gene(s) responsible for processing of rRNA precursors on human chromosomes all hybrids examined contained chromosomal rearrangements and it was impossible to assign the gene(s) involved in the complementation of the *ts422E* defect.

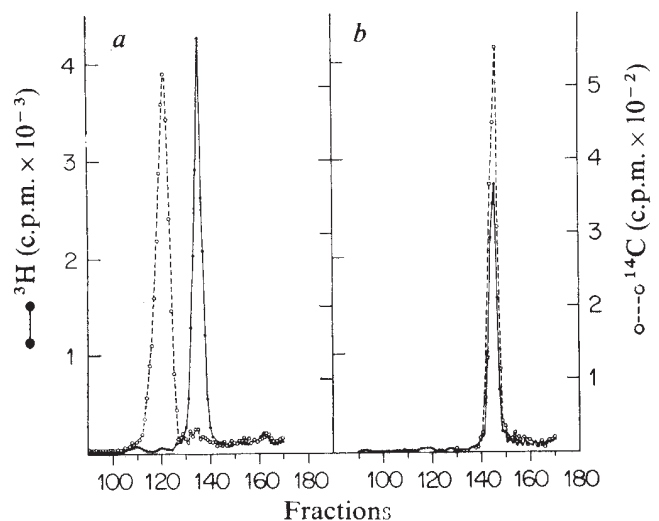
The imperfect complementation can be explained by

assuming that, although the rRNA processing machinery can act on RNAs from related species as efficiently as on RNA from the same species², in phylogenetically distant species such as man and hamster the sites of recognition of rRNA diverged during evolution. This could result in inefficient recognition or binding of accessory preribosomal proteins to the rRNA precursors¹⁵. In this respect it should be remembered that 53-99 C6 hybrid was selected and isolated at the temperature restrictive for the parental mutant. It is thus reasonable to assume that the level of complementation found in the hybrid is the best the population could attain under the pressures imposed.

Why did no human 28S RNA appear at either temperature in the hybrid, resembling the situation found¹⁶ in human-mouse hybrids? Among the hypotheses considered¹⁶ the most probable is the repression of human rRNA genes due to the presence of mouse nucleolar organisers. The situations in the human-hamster hybrid 53-99 C6 could be similar, but the presence of some human 28S rRNA in hybrid populations F3 and F31 suggests that this is not compulsory. It is possible, in our case, that the disappearance of human rRNA during serial culture was due to loss of human rDNA genes.

It seems unlikely that poor complementation is due to insufficient human gene products in the hybrid. The selection of hybrid lines at 39 °C, and in particular their poor

Fig. 3 Plates containing hybrid 53-99 C6 were incubated at 33 °C for 6 d and labelled for 11 h with ³H-uridine (4.4 μCi, 2 μg ml⁻¹). 28S rRNAs were prepared and analysed by polyacrylamide gel electrophoresis in the conditions given in Fig. 1. Electrophoreses were run for 24 h at 5 mA per gel. Samples were run with ¹⁴C-labelled markers from BHK or HeLa cells. *a*, 28S rRNA (³H) from hybrid 53-99 C6 (●); HeLa 28S ¹⁴C RNA (○); *b*, 28S rRNA (³H) from hybrid 53-99 C6 (●); BHK 28S ¹⁴C RNA (○).

**Table 2** Distribution of radioactivity in different species of rRNA in *ts422E*-human hybrids

Cell line (temperature)	32S	28S	18S	Ratio 32S:18S	Ratio 32S:28S	Ratio 28S:18S
53-99 C6 (39 °C)	4.4×10^4	2.2×10^5	3.0×10^5	0.15	0.20	0.72
53-99 C6 (33 °C)	2.8×10^4	2.8×10^5	1.8×10^5	0.16	0.10	1.57
<i>ts422E</i> (39 °C)	9.1×10^3	1.2×10^4	6.1×10^4	0.15	0.76	0.20
Wild-type BHK21 (39 °C)	3.2×10^2	3.6×10^4	1.6×10^4	0.02	0.01	2.25

Cells were labelled for 11 h with ³H-uridine (4 μCi, 2 μg ml⁻¹) and fractionated as explained in Fig. 1. Nucleolar fractions were prepared as previously described⁴, and nucleolar RNA was analysed by polyacrylamide gel electrophoresis in the conditions given in Fig. 1. Nucleolar RNA electrophoreses were run for 9 h. Cytoplasmic RNAs were analysed as in Fig. 2. The data are expressed as c.p.m. per 10⁶ cells.

growth at 39 °C, should have strongly favoured cells which had not lost the gene(s) complementing the *ts422E* defect.

It is difficult, however, to rule out the possibility that human chromosomes are lost continuously in the hybrid populations at 39 °C. Thus each population of hybrid cells, at any given moment, could be a mosaic of cells which have and have not retained the human gene(s) required for normal rRNA maturation and cell growth at the non-permissive temperature. Since data on rRNA production show that about 80% of the cells would have to be mutant-like to produce the observed results, this hypothesis implies that at every cell division the hybrids yield a high proportion of cells behaving as the parental *ts* mutant, and thus unable to grow at 39 °C. Such a mechanism would cause the rate of growth of the cell population to appear linear, rather than exponential, but the rate of growth of the hybrids at 39 °C was exponential (Table 1). We therefore believe this explanation unlikely.

Our data suggest that the human wild-type allele is dominant over the *ts422E* mutation, as it can process hamster rRNA precursors; this processing is, however, inefficient probably due to evolutionary divergence of the rRNAs of the two species.

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Cell density dependence of oscillatory metabolism

THERE is increasing evidence¹⁻³ that the behaviour of cell populations is determined not only by the behaviour of the individual cells, but also by their mutual interactions. One influence on the strength of these interactions may be cellular proximity which, in turn, is a function of cell density. For example, myoblasts fuse only when they reach a critical cell density⁴. The oscillatory glycolytic metabolism of the yeast *Saccharomyces carlsbergensis* has provided a convenient system for the exploration of several biochemical phenomena⁵. For example, whereas yeast cells are generally considered to be metabolically non-interacting, a coupling process of unknown character causes metabolic synchronisation of all cells to a common oscillatory fre-

quency and phase⁶. A suspension of metabolically oscillating yeast therefore behaves as a population of strongly interacting units. Because continuous monitoring of amplitude and phase by fluorometry provides a constant assay of metabolic dynamics, any perturbation of these dynamics is easily recognised as a deviation from the predictable waveform. We describe here modifications in the metabolic behaviour of intact cell suspensions achieved by manipulating the strength of their interaction through changes in cell density.

S. carlsbergensis was grown, collected and assayed for oscillatory metabolism as described before⁷. The results of one experiment are shown in Fig. 1; Fig. 1a records, for a period of 8 min in an oxygenated 0.0125% (w/w) yeast suspension, the total culture fluorescence, which correlates with the intracellular concentration of NADH. Addition of 100 mM glucose at arrow 1 caused a brief surge of intermediates through glycolysis as shown by the increase in NADH concentration preceding the aerobic steady state. On addition of 1 mM KCN to block respiration, (arrow 2) a marked increase in NADH concentration initiated a damped oscillatory approach to the metabolically anaerobic steady state. The second tracing (Fig. 1b) is similar except that the initial density of the cell suspension was 4% (320 times that in the first experiment). At arrow 3 buffer was added and caused a rapid change of suspension density to 2.66%. After a short lag during which the sensitivity of the fluorometer was increased to compensate for the decrease in cell density, metabolic oscillations continued. When this experiment was repeated, but with an initial cell density of 0.0125% (Fig. 1c) rapid density reduction (arrow 3) abolished further detectable oscillatory expression. The

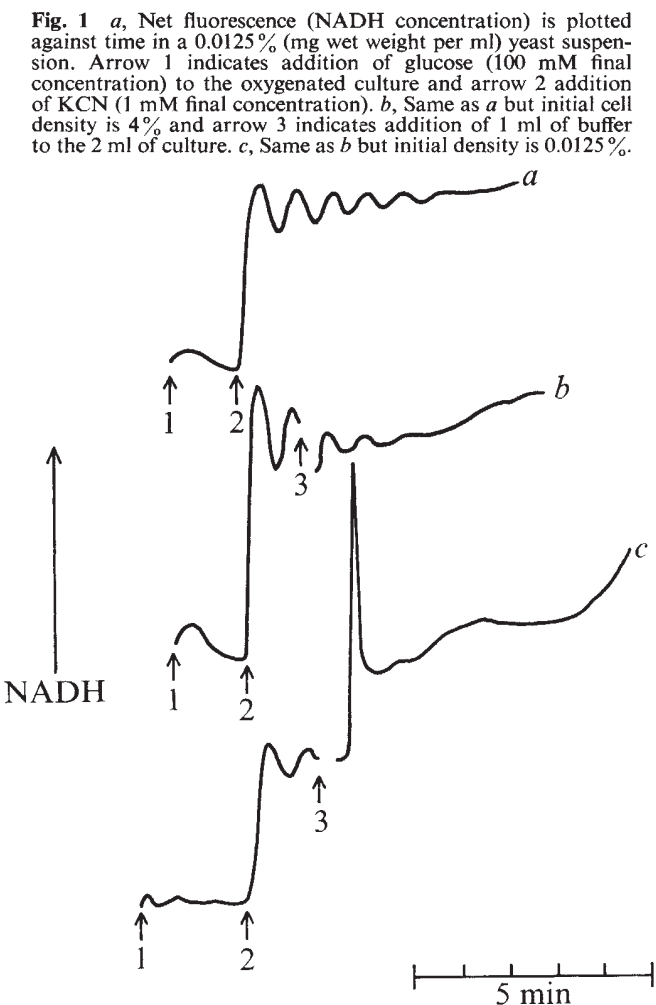


Fig. 1 a, Net fluorescence (NADH concentration) is plotted against time in a 0.0125% (mg wet weight per ml) yeast suspension. Arrow 1 indicates addition of glucose (100 mM final concentration) to the oxygenated culture and arrow 2 addition of KCN (1 mM final concentration). b, Same as a but initial cell density is 4% and arrow 3 indicates addition of 1 ml of buffer to the 2 ml of culture. c, Same as b but initial density is 0.0125%.

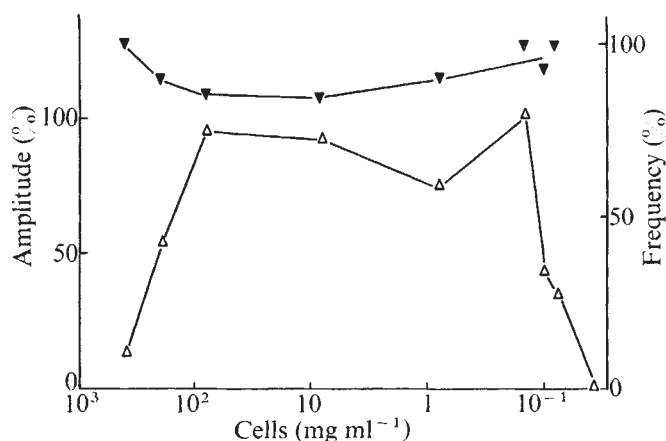


Fig. 2 Normalised amplitude and frequency are plotted against culture density (see text). 100% amplitude corresponds to 100 μ M NADH and the normalisation standard used was a 400 mg ml^{-1} suspension. 100% frequency corresponds to $1/38 \text{ s}^{-1}$. $\blacktriangledown$, Normalised frequency; $\triangle$, normalised amplitude.

sharply peaked transient following density change was a constant characteristic only of oscillatory abolition, and was never shown by suspensions which continued to oscillate. We found that, varying with time of collection, some critical initial cell density could always be found below which no oscillatory metabolism was detected after rapid reduction of cell density.

Figure 2 plots amplitude and frequency against cell density in one such batch. The fluorometer was calibrated by adding known concentrations of NADH to non-metabolising cell suspensions of various densities. Suspensions of equivalent densities then were run as in Fig. 1a. Amplitude was assayed by measuring the height of the second peak from a line connecting the first and third troughs. This distance remained constant, within $\pm 5\%$, in multiple identical experiments. If cell density did not affect the dynamics of each cell, a doubling in cell concentration would be expected to produce a doubling in recorded amplitude. Thus, taking the change in NADH concentration of the second peak in a 400 mg ml^{-1} suspension as a normalisation standard, the height in an 800 mg ml^{-1} suspension was divided by 2 and that in a 200 mg ml^{-1} suspension by 0.5 to correct the raw data for expected density-induced changes in recorded amplitude. We assumed that identical normalised amplitudes should result if no change in dynamics resulted from cell density differences. In Fig. 2 normalised amplitude is expressed as a percentage of the largest second peak amplitude observed in any experiment in the series, and frequency is similarly expressed. Frequency changes little within the range of cell densities tested but amplitude reaches a broad maximum at intermediate densities. This agrees with the finding (Fig. 1) that low cell densities cause a loss of oscillatory metabolic expression. It also suggests that high cell densities inhibit oscillatory expression.

Two explanations for these findings seem possible. Either each cell is affected by cell density and loses its oscillatory capacity, or the population loses synchrony. Measurements made on the total suspension cannot distinguish between these two possibilities, although asynchrony is much harder to rationalise at high densities, when tight coupling might be assumed, than at low densities. Microspectrophotometric measurements⁸ showed previously that individual cells, when oscillating, display both the frequency and waveforms similar to those expressed by the total cell suspension. Although single-cell measurements were not designed to quantify density dependence, there was some indication that a minimal density was necessary to observe single-cell oscillations. These data support the possibility that the inhibition of metabolic oscillations observed at low densities

is not caused by population desynchronisation but by alterations in the stability properties of each cell's metabolic dynamics; that is the steady state of each cell changes from an oscillatory to a non-oscillatory metabolism.

Although the mechanism by which cells synchronise their metabolism is unknown, one might expect its efficiency and characteristics to be a function of cell density and thus underlie the phenomena reported here. This suggests that short range, intercellular communication mechanisms strongly influence not only the quantitative, but also the qualitative metabolic behaviour of cells, such as oscillatory and non-oscillatory behaviour. Furthermore, metabolic synchronisation and density dependence lead to a situation in which the behaviour of the total cell population could be quite unrelated to the sum of the behaviours displayed by individual cells in isolation: that is, biochemical synergism occurs.

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Carcinogenic nitrosamides and cell cultures

THE acylnitrosamides ethylnitrosourea (ENU) and methylnitrosourea (MNU) are potent carcinogens in a variety of animals. Rats, especially, provide a useful experimental system since a single dose administered at the perinatal age can be tumorigenic and in certain strains the nitrosamides in these conditions are relatively site specific in that they produce a high proportion of neural tumours^{1–6}. Although these tumours rarely become clinically apparent before six months, the short term effects of nitrosamides have been studied both *in vitro* and *in vivo* in an attempt to elucidate the oncogenic mechanism. Thus histological examination of tissues from rats recently treated with nitrosamides has revealed a toxic effect on dividing cells⁷ while the use of cell cultures has shown an effect on DNA synthesis and the cell cycle⁸. It has been assumed that both the carcinogenicity and the short term effects occur as a result of the alkylation of macromolecules, in particular DNA⁹. Earlier reports concerned the nature of the chemically modified bases^{10,11} whereas more recently the turnover of such bases has become the focus of attention^{12,13}. We present evidence that the short term effects can be explained by an alternative mechanism to the alkylation of cellular macromolecules; cyanate ions are shown to be a product of MNU and ENU breakdown and it is the cyanate ion which is responsible for short term effects and cytotoxicity.

Cell cultures were derived from the neural tissue of AS rat foetuses on day 18 of gestation, and during studies into the effect of dose and exposure time, chemical breakdown of nitrosamides was followed spectrophotometrically since they are known to decompose rapidly in physiological conditions¹⁴. Figure 1 shows the rate of decomposition in cell culture conditions and the effect of exposure time on cell survival. Since the nitrosamides decay rapidly, yet long

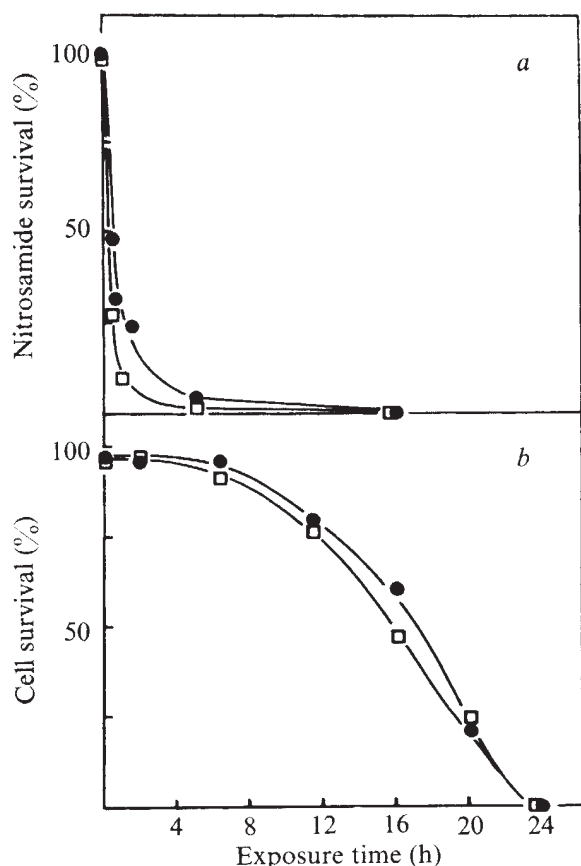


Fig. 1 *a*, MNU ($\square$) or ENU ($\bullet$) were taken up into warm ethanol at 40 mg ml^{-1} and immediately added to Ham's F10 growth medium containing 10% FCS to give a final concentration of 1 mM. At intervals, aliquots of nitrosamide-containing medium were taken and difference-spectra obtained to assess the rate of decomposition. MNU has a peak at 380 nm and ENU at 375 nm. *b*, Primary cultures were set up from the neural tissue of AS rat foetuses on day 18 of gestation. 0.05% trypsin in phosphate-buffered saline (PBS) was used for 15 min to disperse the tissue after sterily removed brains had been very finely minced with scissors. The suspension obtained from one foetal brain was added to F10 medium with 10% serum and then plated out into three 90-mm Petri dishes. In one week such dishes usually achieved confluence. Cells were used before the third passage when a number of different cell types were still present. MNU ($\square$) or ENU ($\bullet$) were added to culture medium as in *a* to give a final concentration of 1 mM and this medium was immediately added to 50 mm plates of cells. At intervals, duplicate plates were washed twice and then incubated with untreated growth medium. At 30 h, all plates were viewed under an inverted microscope with phase-contrast optics. Healthy and lysed cells were easily distinguishable; > 200 cells were counted per plate and cell survival expressed as a percentage.

exposure times bring about cell death, a breakdown product is likely to be responsible for such cytotoxicity. This was confirmed by incubating either nitrosamide at 37°C in growth medium for 3 d before adding the medium to cell cultures when it was found to be as toxic as medium containing freshly-prepared nitrosamide. To identify the breakdown-product, ^{14}C -methylnitrosourea and methylnitroso- ^{14}C -urea were synthesised. Paper chromatography with a solvent system of ethyl acetate-ethanol-water (80:15:5) was used to monitor breakdown of the radiolabelled nitrosamide. When methylnitroso- ^{14}C -urea decomposed in growth medium, the R_f of the isotope changed from 1.0 to 0.26; this product was not urea or methylurea but when $\text{K-}^{14}\text{CNO}$ was applied, the ^{14}C cochromatographed with the breakdown-product. When ^{14}C -methylnitrosourea decomposed the isotope was lost from the R_f 1.0 position, but did not appear elsewhere; this suggested a volatile breakdown product and it was shown by co-distillation with unlabelled material to be methanol. It was thus demonstrated that

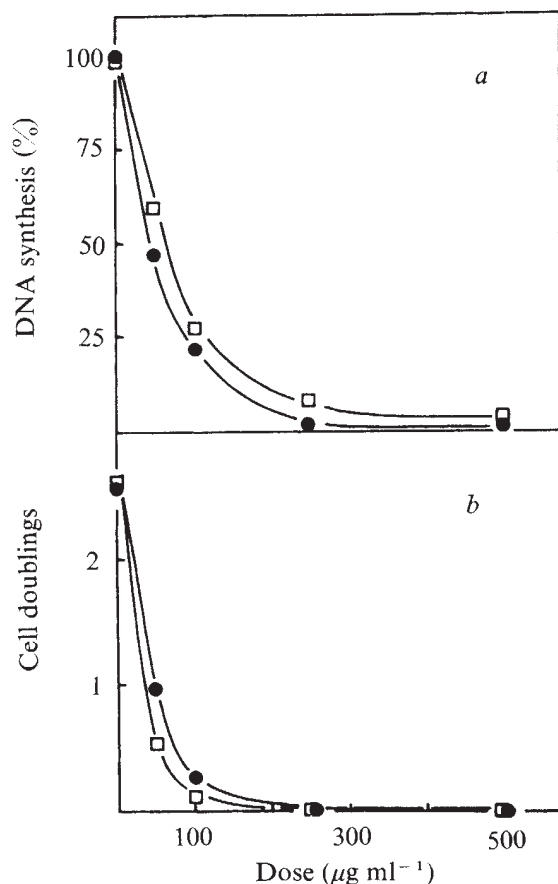
>97% of MNU breaks down in culture conditions, to yield methanol, cyanate ions and gaseous nitrogen.

Cyanate ions were the only possible cytotoxic agent and indeed the cell death induced by nitrosamides is mimicked by potassium cyanate at 0.6 mM. Ethylnitroso- ^{14}C -urea was synthesised and this also yields ^{14}C -cyanate ions when incubated in growth medium. The presence of protein seems to catalyse the breakdown; for example at 37°C the half life of ENU in physiological saline is 150 min, but this goes down to 15 min in growth medium containing serum.

At levels of nitrosamide below 0.3 mM, DNA synthesis and cell proliferation are affected but the cells remain viable. Figure 2 shows how potassium cyanate also mimics these effects.

The cyanate ions are effective by virtue of their ability to effect carbamylation reactions. When cell cultures are incubated with MNU or ENU radiolabelled with ^{14}C in the urea moiety, then the appearance of isotope in the TCA-insoluble material can easily be followed and is still increasing in a linear fashion after 6 h. In contrast, when cells are incubated with MNU of an identical specific activity, but labelled in the alkyl moiety, isotope incorporation into

Fig. 2 Cultures of foetal brain cells were set up as in Fig. 1. Before achieving confluency, 50-mm plates were refed with F10 medium containing 10% FCS and immediately treated with KCNO ($\bullet$) or ENU ($\square$) previously dissolved in warm ethanol. All plates received an identical volume of ethanol. *a*, 10 h after the addition of nitrosamide, triplicate plates at each nitrosamide concentration were pulsed for 1 h with $0.2 \mu\text{Ci ml}^{-1}$ ^3H -thymidine ($>20 \text{ Ci mmol}^{-1}$). After 3 washes with PBS, cells were scraped from the plate with a silicone policeman and treated with a 5% TCA solution. After a further wash with 5% TCA, the insoluble material was collected on a GFC filter. TCA-soluble counts did not vary between plates. The filters were counted in a liquid scintillation spectrometer (Tracerlab); the scintillant was 5 g PPO and 20 g of naphthalene in 1 l of dioxan. *b*, Duplicate plates of cells were set up as in *a* and after 4 d cells were removed by trypsinisation and the number of cells counted with a Coulter counter.



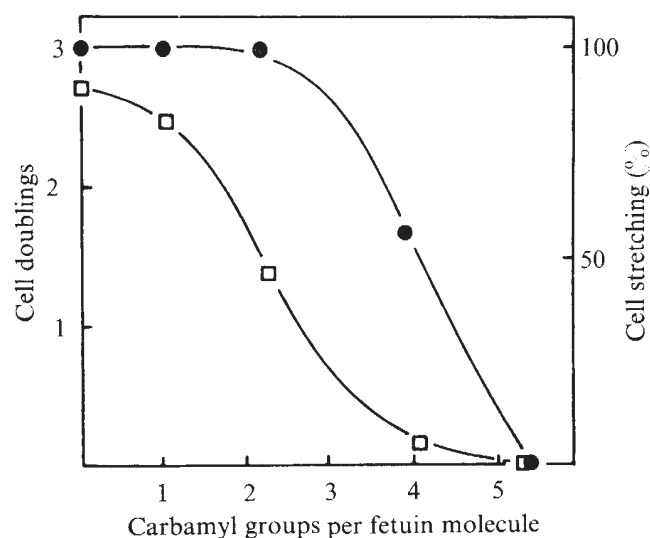


Fig. 3 Fetuin (Sigma) at 15 mg ml^{-1} in PBS was treated with a solution of KCNO (100 mg ml^{-1}) to give a range of final concentrations of KCNO from 0 to 10 mg ml^{-1} . All solutions were made $0.05 \text{ } \mu\text{Ci ml}^{-1}$ with respect to K^{14}CNO and solutions were incubated at 37°C for 5 h. Before and after the incubation, samples were taken and from the TCA soluble and TCA-insoluble counts the amount of carbamylation per mg protein was calculated. This is expressed in terms of carbamyl groups per fetuin molecule. Although gel electrophoresis reveals the presence of contaminating bands, a molecular weight of 46,500 has been assumed. The protein samples were exhaustively dialysed against PBS. Confluent plates of BHK cells were trypsinised and suspended in serum-less F10 and plated-out into 50 mm Petri dishes. The carbamylated fetuin preparations were then added to duplicate plates to give a final concentration of 5 mg ml^{-1} . After 16 h cells were viewed under an inverted microscope with phase contrast optics and cells were counted as "stretched" (that is, having adopted a bipolar morphology) or "unstretched" (that is, totally, spherical). Results are expressed as a percentage of stretched cells (●). After 3 d further plates were trypsinised and counted with a Coulter counter (□).

TCA-insoluble material is at least one order of magnitude lower. It was originally expected that this latter would reflect a low level of alkylation, but it was subsequently found that isotope incorporation was identical if the ^{14}C -methylnitrosourea was first incubated in growth medium for 3 d. Thus it is more likely to reflect low level metabolic utilisation of methanol formed during decomposition.

While the amount of cell-associated protein in a 50-mm dish is usually $50\text{--}75 \text{ } \mu\text{g}$ at confluency, the protein in the growth-supporting foetal calf serum (FCS) will be at least $15,000 \text{ } \mu\text{g}$. Carbamylation of serum proteins by nitrosamides or cyanate also takes place and a correspondingly higher number of counts become associated with serum proteins

than with cellular proteins. This carbamylation of serum protein was found to be very important since it could completely inactivate the serum in respect of its growth-promoting properties. Thus FCS treated at 37°C with 10 mg ml^{-1} KCNO for 4 h followed by exhaustive dialysis produces a chemically modified serum which fails to stimulate DNA synthesis and cell proliferation. FCS is an heterogeneous mixture of proteins and for this reason it is impossible to correlate loss of biological function with degree of carbamylation. We had previously shown¹⁵, however, that certain lines of established cells would grow well with a commercial preparation of the glycoprotein fetuin¹⁶ as sole macromolecular source. BHK cells¹⁷ grow well with 5 mg ml^{-1} fetuin. If the fetuin was carbamylated with ENU, MNU or cyanate, such growth-promoting activities were lost. Using radiolabelled cyanate, the loss of biological activity and the degree of carbamylation were compared (Fig. 3). Above three carbamyl groups per fetuin molecule, BHK cells show startling morphological changes.

BHK cells divide very rapidly and this allowed the demonstration of a very rapid effect of cyanate or nitrosamide. If the cells were in exponential growth then as little as 15 min exposure to one of the above agents could cause an inhibition of mitosis. Table 1 shows such an inhibition achieved with cyanate.

In the past, little attention has been paid to the fate of any part of the ENU or MNU molecule other than the alkyl moiety. But in the case of the more complex nitrosamides, some of which have proved useful chemotherapeutic agents, it has been shown that carbamylation of macromolecules by isocyanate species takes place¹⁸⁻²⁰. Certainly in the case of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, carbamylation of protein has been shown to be a far more extensive reaction than alkylation of macromolecules^{19,20}.

This communication presents evidence that MNU and ENU, on decomposition in culture, give rise to a very bioactive molecule, namely the cyanate ion, which can affect several stages of the cell cycle. The effect of nitrosamides and cyanate on serum indicates that low levels of protein modification may alter biological function. This might also be true inside the cell and in view of its activity *in vitro*, it is now necessary to determine whether the cyanate ion is responsible for any of the effects of nitrosamides *in vivo*.

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Table 1 Inhibition of mitosis of BHK cells by cyanate

Time of exposure to KCNO (min)	Increase in cell no. per plate $\times 10^{-5}$ after:			
	2 h	4 h	6 h	24 h
0	0.8	2.0	3.5	14.4
15	0	0.4	2.3	14.0
60	0	0	0.5	11.6

Replicate plates of BHK cells in exponential phase of growth were treated with 0.5 ml of a KCNO solution in PBS to give a final concentration of 0.4 mg ml^{-1} . At the end of the exposure-time cells were washed and refed with ordinary growth medium. Control cultures were treated with a similar volume of PBS and washed and refed after 1 h. At 1 h ($t=0$) and intervals thereafter, duplicate plates were trypsinised and counted with a Coulter counter.

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Isolation of immunogenic tumour cells by cell-affinity chromatography

TUMOUR-HOST relationships are determined by many variables^{1,2}, and may be altered to favour the host by the use of chemically modified cells which act as specific immunostimulants³. Furthermore, modified cells have therapeutic value in mice with small tumours^{4,5}. Several studies have shown that conventional methods followed by administration of immunostimulants are very effective in treating tumours and inducing tumour-specific immunity⁶⁻⁸. Combined chemotherapy and immunotherapy of L1210 leukaemia with neuraminidase-treated (VCN) tumour cells yielded a higher percentage of long term survivors than did chemotherapy alone^{6,7,9,10}. The therapeutic effectiveness of VCN-treated cells depended on several variables¹⁰, including proper selection of immunogenic cells^{7,11}.

We now report that highly immunogenic cells can be isolated from the parental tumour cell population by cell-affinity chromatography with concanavalin A (con A). Immunisation with these cells after chemotherapy resulted in complete remission of L1210 leukaemia.

L1210 cells (Arthur D. Little Co.) were maintained as an ascitic tumour in BDF₁ (C57BL/6×DBA/2) males and athymic (*nu/nu*) mice. The cell line has also been maintained as a suspension culture using RPMI 1640 medium, supplemented with 5% foetal calf serum and antibiotics.

Cell-affinity columns were prepared by covalent coupling of con A to nylon fibres with glutaraldehyde¹². L-leucine was added to the reaction mixture to compete with, and thereby "space" the con A molecules along the fibre. This technique resulted in a fourfold decrease of bound protein compared with coupling in the absence of the amino acid. The preparation and properties of the con A columns will be outlined in detail elsewhere. Recognising that the binding of cells to the con A-nylon was probably mediated through multiple (and different) sugar linkages, it was decided to elute the cells from the column by the sequential addition of sugars that possessed a decreasing order of free-solution affinity for con A¹³. The elution profiles for L1210 cells grown in the BDF₁ host, in culture, or in the athymic host are summarised in Table 1. The two cell lines grown *in vivo* seemed to represent the extremes in chromatography, since few cells were released by sugars, and the cell lines were primarily composed of cells either binding or not binding to the con A-nylon.

The immunogenic properties of the isolated subpopula-

Table 1 Subpopulations of L1210 tumour cells (%) as a function of growth conditions

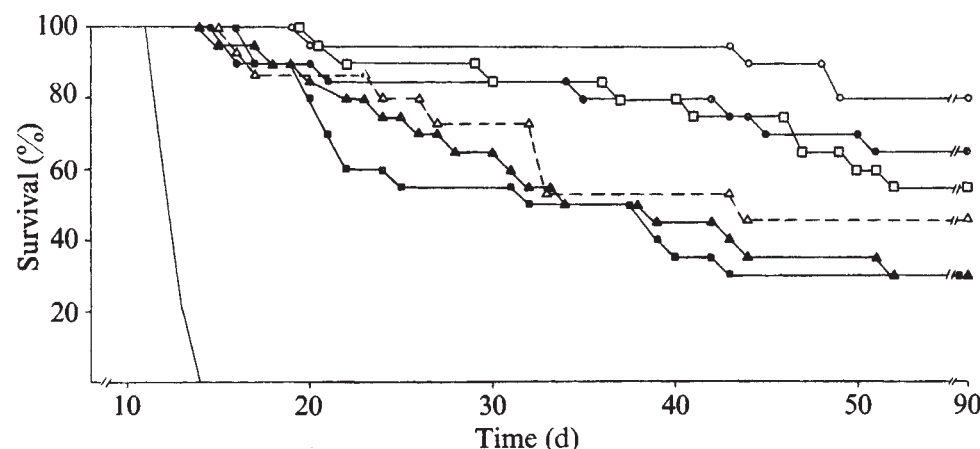
Cell fractions	<i>In vivo</i> (BDF ₁)	Growth conditions	
		<i>In vivo</i> (athymic)	<i>In vitro</i>
A	14.1	68.0	32.0
B	4.1	0.6	5.0
C	<0.01	0.2	0.7
D	<0.01	0.2	2.8
E	<0.01	0.03	3.9
F	81.8	30.6	56.6

Fractions A to F represent the percentage of total cells. A, eluted with phosphate-buffered saline (PBS); B, eluted with α -methyl-D-mannoside; C, eluted with α -methyl-D-glucoside; D, eluted with sucrose; E, eluted with α -D-glucose; F, not eluted by the above sequence. Cells (ascitic tumours) grown *in vivo* were washed once in 1% ammonium oxalate, 25 °C, to lyse erythrocytes, followed by two washes in PBS. Cultured cells were washed twice in PBS. Preparation of *in vivo*-grown cells by Ficoll-Hypaque gradients³⁵ did not affect the elution profile. Affinity columns were prepared by packing 25-cm glass pipettes with brushed nylon (No. 3 denier, 14 inch, type 200, DuPont) then incubated with equal volumes of 25% glutaraldehyde, 0.4 μ M L-leucine, and 1 mg con A (con A was Grade III, twice crystallised, all reagents Sigma), at pH 6.5 for 1 h at 37 °C. This procedure bound 1.9 μ g protein per g nylon. The column was then flushed with 50 volumes PBS, pH 7.2. Elution was at room temperature with a flow rate of 2 ml min⁻¹.

tions were evaluated by their effectiveness in causing complete remission of L1210 tumour. The optimal protocol for this particular tumour-host model was developed by Kollmorgen *et al.*¹⁰ and is based on initial reduction of tumour burden by chemotherapy. Immunotherapy consisted of a single injection of one of the different cell fractions. Survival after therapy correlated with the elution profile from the con A column (Fig. 1) with the best response observed in mice treated with fraction A. All surviving mice were immune to L1210 tumour when rechallenged with a lethal inoculum for virgin mice.

To further evaluate the immunogenic properties of these cells, the best and worst fractions (A and E, respectively) were treated with neuraminidase and used in an identical protocol. As Fig. 2 shows, the relative effectiveness of the two cell types was reversed after treatment with VCN, although long-term survival was similar. The early deaths observed in the group of mice immunised with VCN-treated fraction A cells suggests that chemotherapy with 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) had an immunological component which was abrogated, in this case, by immunotherapy.

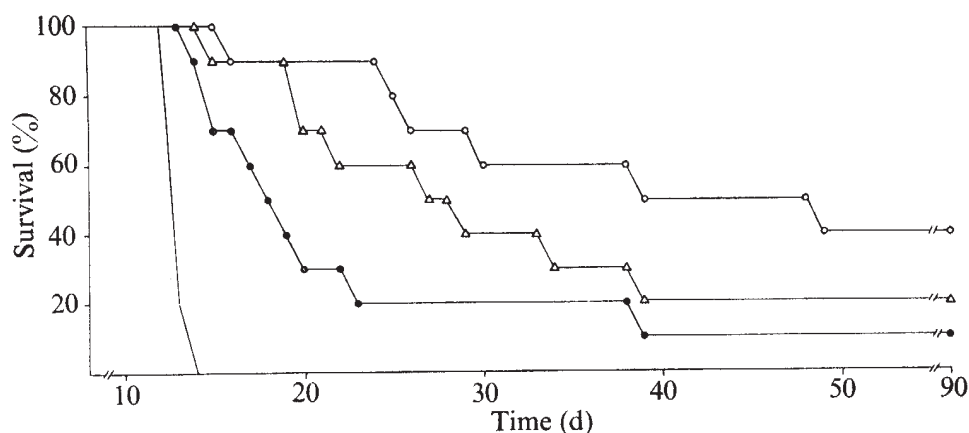
These results indicated that the immunogenicity of the different subpopulations was related to the expression of cell surface carbohydrates, as defined by con A-affinity



solid line indicates the survival of tumour controls (no treatment). Δ , Chemotherapy with BCNU only; $\circ$, BCNU + fraction A; $\square$, BCNU + fraction B; $\bullet$, BCNU + fraction C; $\blacktriangle$, BCNU + fraction D; $\blacksquare$, BCNU + fraction E.

Fig. 1 Percentage survival as a function of time after tumour injection. This protocol is based on optimisation of the L1210-BDF₁ tumour-host model¹⁰ where mice received an intraperitoneal injection of 10⁴ cells at time zero. The mice were randomised on day 6.5 (estimated tumour burden 10⁴ cells) and all but tumour controls received a single intraperitoneal injection of BCNU (12 mg kg⁻¹) to reduce tumour burden. The mice were again randomised on day 8, and immunotherapy consisted of a single intraperitoneal injection of 10⁴ cells (inactivation of the cells by one freeze-thaw generally resulted in less than 10% viability). Each group contained 20 mice. The

Fig. 2 Percentage survival as a function of time after tumour injection. The experimental protocol is identical to that described in Fig. 1, except that fraction A and fraction E cells were treated with VCN (Grand Island Biological). The cells were concentrated by centrifugation and incubated with equal parts acetate buffer, pH 5.6 and VCN (1 U per 10^6 cells) for 1 h at 37 °C. The cells were washed twice with 5 mM EDTA-saline and stored in PBS at -90 °C until use. Each group contained 10 mice. The solid line indicates the survival of tumour controls (no treatment). Δ , Chemotherapy with BCNU only; $\circ$, BCNU + VCN-treated fraction E; $\bullet$, BCNU + VCN-treated fraction A.



chromatography. Further studies were designed to examine the nature of L1210 cell antigenicity. Mice were immunised with VCN-treated, cultured L1210 cells in the combination therapy protocol previously described. The survivors were boosted with 10^6 viable L1210 cells (to which they were immune) and bled 7 d later. The serum was then tested for complement-dependent cytotoxicity against cultured L1210 cells, in the presence of absence of various sugars. Cytotoxicity was measured by the ^{51}Cr -release assay¹⁴, and the results are expressed as mmol of sugar required to cause 75% inhibition of cytotoxicity (Table 2). Of the sugars used to elute cells from the con A column, the most effective inhibitor of cytotoxicity was α -methyl-D-mannoside, the sugar which released fraction B. The poorest inhibitor was α -D-glucose, which released fraction E. Galactosyl-like residues have been considered as part of the binding site for "naturally-occurring" cytotoxic antibodies to VCN-treated cells^{15,16}. Galactose and galactosamine were non-inhibitors in the study reported here, although the non-reducing sugar α -methyl-D-galactoside was an effective inhibitor of cytotoxicity. Maximal inhibition of cytotoxicity was observed using fucose. Preliminary studies have shown that the percentage of cells released from fucose-specific lectin columns was three to five times higher for fraction A than for the whole tumour cell population (J.J.K., unpublished). Experiments are in progress to evaluate the immunogenic properties of tumour cells isolated by fucose-specific affinity columns.

Edelman *et al.* have shown that antigen-specific lymphoid cells can be isolated by their binding to antigen-derived

fibres¹⁷. The use of their techniques¹⁷⁻¹⁹, in combination with the chromatography of tumour cells described here, offers an excellent system from study of interactions between lymphoid and tumour cells.

Tumour cell populations are heterogeneous with respect to various biological²⁰⁻²² and antigenic properties^{23,24}. This heterogeneity may provide clones with different membrane characteristics and nutrient requirements, and thereby influence the rate of tumour growth and extent of metastatic involvement. Metastasis may be mediated by cell adhesion²⁵, which in turn, may be a function of cell surface proteases, glycosidases and charge density²⁶⁻²⁸. Cellular interaction of blood-borne tumour cells with lymphocytes²⁹ and platelets³⁰ can also affect the distribution of circulating tumour cells. Different growth conditions may also result in the alteration of membrane properties³¹, immunogenicity^{7,32} and metastatic properties of tumour³³.

These observations, together with the results reported here have important implications for the immunotherapy of cancer. We have reported¹¹ that immunotherapy was successful only when cells used for immunisation elicited immunological products that were cytotoxic for residual tumour. Animals with drug-resistant tumour required immunisation with drug-resistant cells for optimal therapeutic benefit (J.J.K., unpublished). Drug-resistant sublines expressed marked alterations in antigenicity³⁴ and serum from mice cured by immunisation with VCN-treated, drug-resistant cells had minimal cytotoxicity directed against the parental cell line¹¹.

In the treatment of human cancer, the probable source of autologous tumour cells for subsequent immunotherapy will be preserved specimens of the primary tumour. These cells may bear little antigenic resemblance to tumour cells which emerge during treatment.

The results reported here suggest that a new approach to active, specific immunotherapy may be developed—to "antigenically match" residual tumour with isolated sub-populations from the primary (parental) neoplasm. This will require that predictive relationships be established between the antigenic properties of metastatic and drug-resistant tumour cells, and the antigenic properties of isolated sub-populations from parental tumour. In L1210 leukaemia, the immunogenic fraction A expanded when the cell line was cultured, and was the dominant cell type when tumour was grown in athymic mice, suggesting an excellent source for immunogenic cells.

Studies are in progress to extend this concept of immunotherapy to other tumour-host models, and to the therapy of antigenically distinct clones of tumour cells that may emerge after treatment with chemotherapeutic agents.

We thank Ms Ann LeFever and Mr Kirt Bierig for technical assistance, and the Experimental Immunotherapy Group, Oklahoma Medical Research Foundation for helpful

Table 2 Carbohydrate-inhibition of complement-dependent serum cytotoxicity to cultured L1210 cells

Inhibiting carbohydrates	mM required for 75% inhibition
α -D-Glucose	50.00
Sucrose	1.00
β -D-Glucose	0.30
α -Methyl-D-galactoside	0.01
α -Methyl-D-mannoside	0.007
α -D-Fucose	0.001
α -D-Fucose	0.001
Non-inhibiting carbohydrates	
D-(+)-Galactose	
D-(+)-Galactosamine	
N-Acetyl-D-glucosamine	
α -Methyl-D-glucoside	

Mice were treated in an identical experimental protocol as described in Fig. 1, except immunotherapy consisted of VCN-treated, cultured L1210 cells. The serum of surviving mice were pooled and cytotoxicity was determined by ^{51}Cr release¹⁴. Serum was preincubated with sugars for 30 min before addition of target cells. Serum cytotoxicity without inhibitor was 40% of total releasable ^{51}Cr , and all inhibitors were tested from 300 mM to 10 pmol.

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Differential response of cells grown in light and dark to near-ultraviolet light

We have previously shown that near-ultraviolet and visible light (290-650 nm) kill mitotic and early S phase amoebae, whereas G₂ phase amoebae are quite resistant¹. Exposure of amoebae to near-ultraviolet light alone (290-400 nm) can have similar results (our unpublished data). We report here variations in sensitivity to near-ultraviolet irradiation of amoebae grown in 12 h light/12 h dark (hereafter referred to as light-grown cells) and those grown in complete darkness. We also show that in the dark-grown cells, as well as the S phase of the light-grown cells, the repair mechanism against the induced lethal damage might be lacking or non-functional.

Amoeba proteus were cultured at 21 ± 1 °C, as before². Both light-grown and dark-grown cells have a generation time of 36 ± 4 h, and an S period of 6-7 h with no G₁ (ref. 3). The dark-grown cells (originally from a light-grown culture) had been kept in an incubator at 20 °C for about 22 months. They were exposed briefly to light when the culture dishes were changed every third day. The intensity of light incident on the light-grown cell cultures was ~ 5 W m⁻² from fluorescent tubes. The amoebae grew equally well when moved from dark to light and vice versa.

Light-grown and dark-grown amoebae were exposed at different phases of the cell cycle to near-ultraviolet irradiation at an intensity of 14 W m⁻² for various periods. The survival curves (Fig. 1) show that both the S and the G₂ stages of the dark-grown cells (DS and DG₂) as well as the S phase of the light-grown cells (LS) were killed within 5-7 d at an energy

fluence of ~ 1.5 × 10⁵ J m⁻² of near-ultraviolet light. This dose also killed all the mitotic cells from light-grown and dark-grown cultures (not shown). In contrast, the G₂ phase amoebae from light-grown cultures (LG₂) were highly resistant to this dose of near-ultraviolet light, although a division delay (between 48-96 h) was noted. In controls, the survival rate was >99%.

To assess the sensitivity of the nucleus and cytoplasm of the amoebae to near-ultraviolet light, nuclear transplantation experiments (according to the method of Jeon and Lorch⁴) were undertaken. Amoebae exposed to the lethal dose (1.5 × 10⁵ J m⁻²) of near-ultraviolet light were selected for nuclear transplantation studies. All nuclear transfers were carried out almost immediately after exposure and completed within ~ 30 min. In most cases, both the nucleus and the cytoplasm of dark-grown and near-ultraviolet-exposed cells failed to survive when recombined with the cytoplasm and nucleus respectively from control LG₂ or DG₂ phase cells. When the nucleus from a light-grown and near-ultraviolet-exposed S phase cell was grafted into the control LG₂ phase cytoplasm a partial recovery (~ 30%) of the treated nucleus was observed. Most of the treated cytoplasm from the light-grown cells could, however, be rescued when the nucleus from control LG₂ cell was implanted into them (see Table 1).

These experiments show that: first, the control LG₂ and DG₂

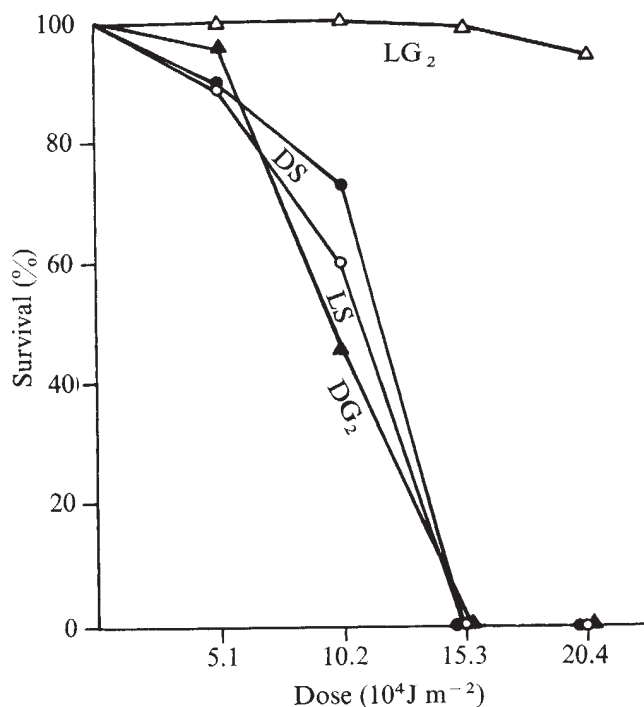


Fig. 1 Survival curve of near-ultraviolet exposed light-grown and dark-grown amoebae at different phases of the cell cycle. LS, LG₂, S phase and G₂ phase of light-grown amoebae; DS, DG₂, S phase and G₂ phase of dark-grown amoebae. The amoebae at known cell cycle stages, (S phase, 0-1-h-old cells; G₂ phase 18-24-h-old cells), obtained by mechanical selection of the division spheres, were placed in a specially constructed watch glass, partitioned into four chambers. A Reichart UG-1 filter with < 1% transmission below 280 nm and above 410 nm, and having a maximum transmission of 73% at 360 nm, was placed on the top of the watch glass. The light source used was a 400-W Philips white fluorescent lamp¹ and the amoebae of different age groups were exposed simultaneously to near-ultraviolet light transmitted through the filter at an intensity of 14 W m⁻² as measured by a Schwarz linear vacuum thermopile. The temperature of the culture medium during the exposure was maintained at ~ 20 °C by means of a water-circulating thermostat (Lauda). The cells were washed after exposure with medium and kept in syracuse watch glasses together with food organisms. No difference in response was noted between cells kept in total darkness and those kept in a 12 h light/12 h dark cycle after the exposure. Between 50 and 150 amoebae were exposed for each experimental set.

Table 1 Results of nuclear transplantation experiments between near-ultraviolet treated and control amoebae from light-grown and dark-grown cultures at different cell cycle stages

Type of transfer	No. of transplants	No. of cell deaths	Survival† (%)
$A(D)S_n^* \rightarrow (D)S_n \leftarrow \} (L/D)G_c$	69 38	67 4	3 90
$(D)S_c^* \leftarrow (D)S_c \leftarrow \} (L/D)G_n$	65 36	59 3	9 91
$B(L)S_n^* \rightarrow (L)S_n \rightarrow \} (L)G_c$	15 17	10 1	33 94
$(L)S_c^* \leftarrow (L)S_c \leftarrow \} (L)G_n$	15 14	1 2	93 86

*Near-ultraviolet treated cells. n_c , c , Indicate the nucleus and the enucleated cytoplasm respectively.

S, S phase (2–3 h after mitosis); G₂, G₂ phase (18–20 h after mitosis). (D), Dark-grown cells; (L), light-grown cells; (L/D), light or dark-grown cells.

†Production of at least sixteen amoebae from a single transplant has been taken as an index of survival.

nuclei cannot repair near-ultraviolet-induced damage of the DS cytoplasm. Second, the control LG₂ nucleus can repair the damage of the treated LS cytoplasm. Third, the treated LS nucleus can partially recover when transferred into the control LG₂ cytoplasm, but the treated DS nucleus cannot recover when grafted into either the LG₂ or DG₂ control cytoplasm.

The cytoplasm and the nucleus of dark-grown cells both seem to be highly sensitive, whereas the nucleus of light-grown S phase cells is partially, and the cytoplasm fully, resistant to near-ultraviolet light. Furthermore, our data strongly suggest the presence of a recovery factor(s), for example, a repair enzyme against the near-ultraviolet-induced damage in light-grown G₂ phase amoebae, which might be lacking or non-functional in the other cases mentioned here (see also ref. 1). It is interesting that an LS nucleus exposed to near-ultraviolet light can partially recover in the control LG₂ cytoplasm, while the treated DS nucleus shows insignificant recovery in either LG₂ or DG₂ control cytoplasm. Thus, it seems that repair against damage induced by near-ultraviolet light involves, as well as a recovery factor(s) in the LG₂ cytoplasm, a capacity of the nucleus of the exposed cell to augment recovery—which is probably absent in the dark-grown cells.

Near-ultraviolet light induces thymine dimer formation, DNA single-strand breaks, and also inactivates photoreactivating enzyme. Because of the poor absorption of DNA and proteins in this region of the spectrum, it has been suggested that the mechanism of induction of such damage is similar to that of photodynamic effect, being mediated through an endogenous sensitizer, for example, riboflavin or ubiquinone (for review see ref. 5).

We suggest that a certain region of the visible light spectrum can repair, directly or indirectly, the damage done by 360-nm light or that it is antagonistic to the deleterious action of this light before the fixation of the damage in the cell. The reasons are as follows: first, cells exposed to near-ultraviolet light are killed much earlier (5–7 d) than those exposed to visible light (3–5 weeks)¹. Second, almost an equivalent dose ($1.5 \times 10^5 \text{ J m}^{-2}$) of 350–370-nm light was received by both the cells exposed to visible light¹ and to near-ultraviolet light, resulting in 100% lethality. At this dose, light-grown S phase nuclei exposed to visible light show almost complete recovery (88%) when transplanted into the control G₂ phase cytoplasm, compared with partial recovery (33%) when exposed to near-ultraviolet light.

There are some reports that growth in darkness has changed or modified cellular functions. *Nicotiana tabacum* plants, kept

in the dark, lose their ability to photoreactivate ultraviolet-treated tobacco mosaic virus RNA within a week. Such plants recover their photoreactivation ability after they are returned to the light⁶. Mutants have been obtained from *Rhodospirillum rubrum* cells, grown for > 100 generations in the dark, which are light-sensitive and produce only a trace amount of bacteriochlorophyll *a* (ref. 7).

Finally, it would be interesting to know the timings of the 'switch over' from resistance to sensitivity to near-ultraviolet light and vice versa in the amoebae. Our preliminary experiments suggest that dark-grown G₂ phase cells acquire resistance to near-ultraviolet light in 10 d, or less, after they are returned to the light. The induction of this resistance thus seems to be photoregulated.

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Herpes simplex virus infection stops spontaneous beating of chick heart cells

PROFOUND modifications in the macromolecular structure of cell membranes and membrane-related functions have been demonstrated on infection with various viruses^{1,2}. For example, shortly after infection with certain herpesviruses, synthesis and insertion of host proteins into plasma membranes cease and new virus-specified proteins begin to appear^{3,4}. These viral proteins differ from the cell membrane proteins in size, electrophoretic mobility, extent of glycosylation and sulphate incorporation^{3–5}. The changes in membrane structure are reflected in altered morphology, antigenic characteristics and contact interactions of herpesvirus-infected cells^{4,6}. They also appear to affect the ion-dependent electrical properties of the cells as shown by alterations in the transmembrane potential of cells infected by herpesviruses *in vitro* and *in vivo*^{7,8}. To elucidate further alterations in membrane-related cell functions, as well as virus-cell interactions associated with herpesvirus infection, we are studying the effects of herpes simplex viruses (HSV) type 1 and 2 on chick embryo heart cells, which provide a useful system, particularly when aggregated into spontaneously beating spheroidal clusters^{10–13}. We found that these viruses stopped the spontaneous beating of the cells before cell 'death'.

Cell monolayers were prepared from 7-d-old chick embryo hearts by a multicycle trypsinisation procedure described before¹⁰. Dishes (35 mm diameter) containing 2 ml of medium 818A were seeded with 2×10^5 cells per dish and incubated at 37 °C in a water-saturated atmosphere of 5% CO₂, 10% O₂ and 85% N₂. Cell aggregates were prepared from the dissociated heart cells by incubating Erlenmeyer flasks (50 ml) containing 10 ml of medium and 1×10^6 cells per flask (gassed with 5% CO₂) at 37 °C on a shaker gyrating at 75 r.p.m. (ref. 11). After 18–20 h of incubation, cultures were washed and examined on the constant-temperature (37 °C) stage of an inverted microscope, in 5% CO₂ atmosphere.

Heart cell monolayers and aggregates were inoculated for most experiments with HSV-1 (VR₃ strain) at multiplicities of infection (MOI) between 0.25 and 200 plaque forming units (PFU) per cell. After 90 min of viral adsorption at 37 °C, the preparations were washed with fresh

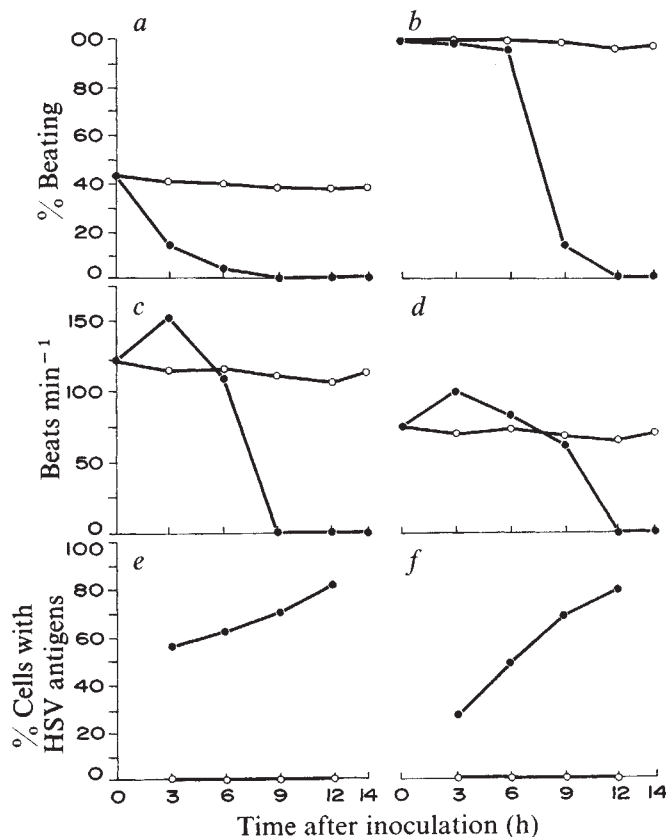


Fig. 1 Embryonic chick heart cell monolayers (*a*, *c* and *e*) and aggregates (*b*, *d* and *f*) inoculated with herpes simplex virus (●) or mock inoculated with inactivated virus (○). *a* and *b*, Percentage of heart cells in monolayers or aggregates beating. *c* and *d*, Beating rate (beats per min). *e* and *f*, Percentage of cells exhibiting HSV antigens by immunofluorescence in monolayer or aggregated heart cells.

medium and reincubated as before. To confirm that cultures were infected, supernatant medium or homogenates of the cells were subinoculated into cultures of primary rabbit kidney cells. In addition, HSV antigens were detected in the virus-inoculated heart cells by a direct immunofluorescent technique¹⁴. Heart cultures mock inoculated with virus inactivated at 56 °C for 3 h served as controls.

HSV infection of heart cells in monolayer culture caused the cells to round up within 6–12 h and stop beating. In a typical test, the number of beating cells in monolayers inoculated with virus (MOI=100) fell from 43% at the time of inoculation to 4% 6 h later (Fig. 1*a*). Only a few cells beat arrhythmically and with variable rates 9 h after inoculation. At 12 h, none were beating; contractions were not restored by a change of medium. No significant alterations were observed in the beating activity of cells mock inoculated with heat-inactivated virus.

When the virus inoculum was added to cell suspensions on a gyratory shaker at the beginning of aggregation, cells either failed to aggregate or formed irregular clumps of loosely attached cells. For this reason, preformed spheroidal aggregates were inoculated with HSV after 24 h of gyration. Rounding of cells occurred at the periphery of the aggregates. At the time of inoculation, 98–100% of the aggregates beat, but only 14% continued beating 9 h later; none of the aggregates beat 12 h after inoculation (Fig. 1*b*). Preliminary recordings of aggregates impaled with intracellular electrodes at intervals after HSV infection indicated that action potentials ceased and membrane resting potential declined slightly when the beat stopped.

The mean beating rate of cells and aggregates increased

by 25–30% over the control rate 3 h after inoculation, and then declined gradually before beating stopped (Fig. 1*c* and *d*). Mock inoculated control aggregates continued to pulsate without significant change in rate. The transient increase in beating rate after virus inoculation resembles the initial increase in beating rate of aggregates partially depolarised by ultraviolet light¹⁵, and may be attributable to depolarisation.

Inoculation with the VR₃ strain of HSV-1 in a range of MOI between 0.25 and 200 PFU per cell demonstrated that an MOI greater than 2.5 was required to stop the beating of either single cells or aggregates within 24 h. Infection with two other HSV-1 strains (F and HEA) at MOI of 100 produced effects on the heart cells similar to those observed with the VR₃ strain, while two HSV-2 strains (MS and G) stopped the beat at the same time with an MOI of 20.

Three hours after inoculation with the HSV-1 (VR₃) at an MOI of 100, viral antigens were detected by immunofluorescence in about half the cells in monolayer culture, when the percentage of beating cells had dropped from 45 to 18% (Fig. 1*a* and *e*). Thereafter, as the number of beating cells continued to fall, there was a concomitant increase in the percentage of HSV antigen-positive cells. In contrast, 95% of the aggregates continued to beat 6 h after inoculation, when about half of their constituent cells exhibited viral antigens (Fig. 1*b* and *f*). The number of beating aggregates declined abruptly to 15% 3 h later, when antigen-positive cells had increased to 70%. The difference in response between monolayer cells and aggregates may be attributed to the depolarisation in the tightly coupled aggregates^{11,12}, as well as to a difference in the rate of viral multiplication in aggregated cells as compared to monolayer cells due to the differences in topography.

It is noteworthy that 12 h after infection, at the time all cells and aggregates had stopped beating, 92% of the cells in monolayers were found to exclude Trypan blue dye. Similarly, when the 12-h-infected aggregates were dissociated with trypsin, 95% of their component cells excluded the dye. This indicates that viral infection at this time had not caused the cell membranes to become permeable to molecules the size of Trypan blue, and suggests further that the loss of beat and electrical activity did not result merely from non-selective leakiness of the cell membranes. Although virus-infected cells eventually die, the brief interval (3–6 h) between viral inoculation and the effects on beating rate reported here argues for more subtle alterations in the infected cell membranes as the underlying cause of these physiological changes. More work is needed to discover how such changes are correlated with those that have been observed with biochemical and immunological methods at the same early time interval^{14,6}.

As far as we know, no earlier investigations of the pathophysiological effects of viruses on embryonic heart cells have been reported, except that such cells infected with mumps virus in conditions somewhat different from those we used, continued beating¹⁶.

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Unrelated animal viruses share receptors

THE specific receptors for several non-enveloped viruses are present on cells in only limited numbers (1×10^4 – 10×10^4), and can therefore be saturated with excess virus¹⁻³. An

excess of inactivated poliovirus type 1 inhibits the attachment of infectious poliovirus of all 3 serotypes, but not the attachment of other viruses, including B Coxsackie viruses⁴. Most of the B Coxsackie viruses probably share the same receptor⁵, but Coxsackie viruses B1 and B3 do not compete for the poliovirus receptor¹. Adenovirus 2 and 5 also appear to share the same receptor, since their attachment to host cells is blocked by an excess of the adenovirus type 2 fibre protein, which seems to be the receptor-recognising portion of the virus particle². A number of other adenoviruses may also belong to the same receptor family since soluble antigens block their attachment to erythrocytes⁶. We here report results which permit the construction of a 'receptor map' for a number of non-enveloped viruses.

These experiments measured attachment of highly purified ¹⁴C-, ³⁵S-, or ³²P-labelled virus particles to HeLa cells. The HeLa cells had either not been exposed to another virus, or exposed to excess purified virus or viral antigen to saturate most of the available receptor sites of one type. Pretreatment with excess virus was usually for 15 min at 34.5 °C

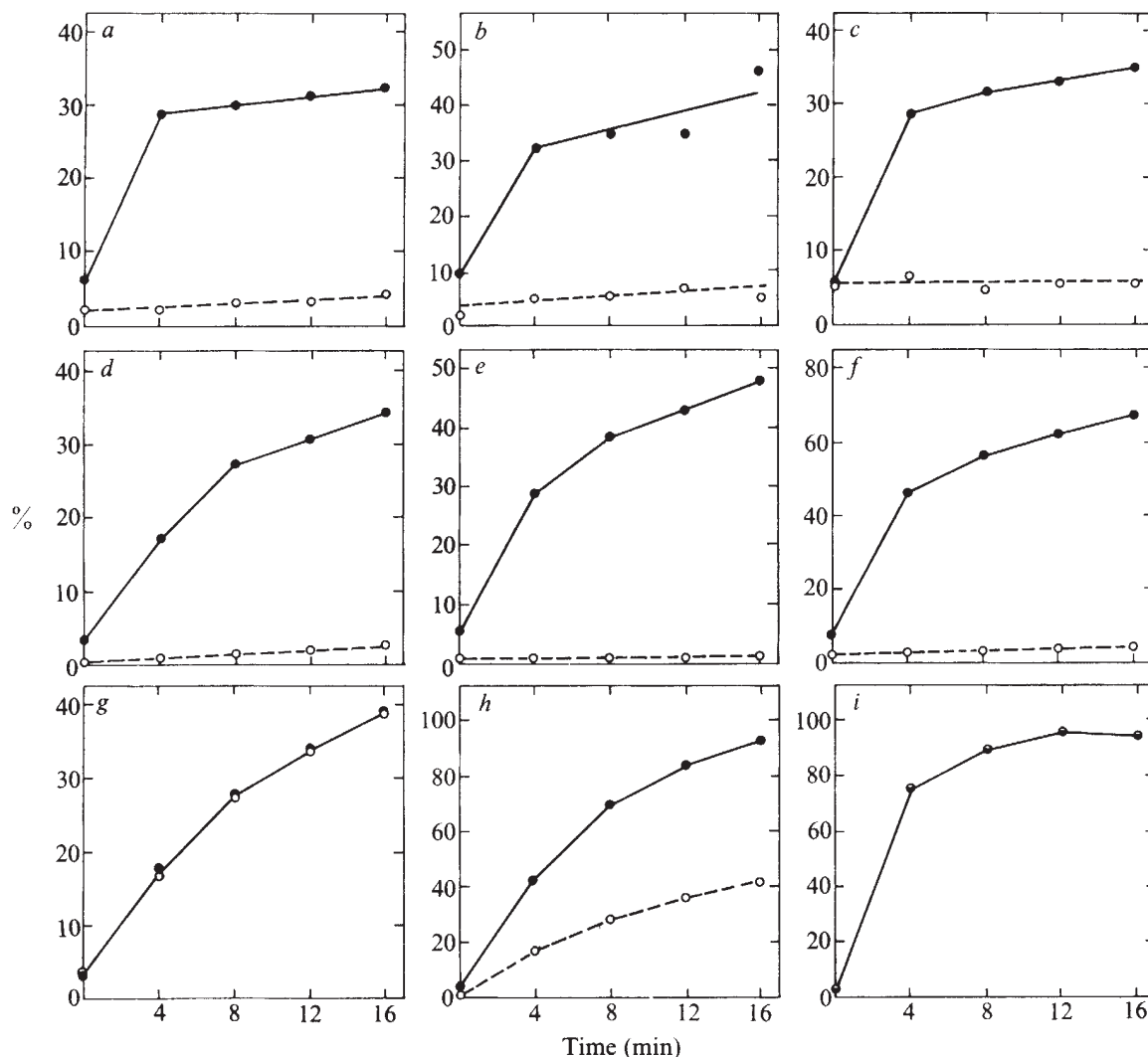


Fig. 1 Attachment of labelled virus particles to untreated cells (●) or to cells treated with excess unlabelled CB3, Ad2 or Ad2-fibre protein (○). Attachment of ¹⁴C-CB3 was inhibited by excess homologous virus (a), and by excess Ad2 (b) or by excess Ad2 fibre protein (c). Attachment of ³⁵S-Ad2 was inhibited by excess CB3 (d), by excess homologous virus (e) or excess fibre (f). CB3 did not inhibit attachment of CA21 (g). Excess Ad2 particles partly inhibited attachment of HRV-2 (h) but the fibre protein did not (i). Non-radioactive virus (1×10^5 – 3×10^6 particles per cell) or Ad2 fibre protein ($8 \mu\text{g ml}^{-1}$) was incubated for 15 min at 34.5 °C with HeLa cells ($1 \times 10^7 \text{ ml}^{-1}$), or cells were 'mock' incubated without added virus or fibre. The suspensions were cooled to 0 °C and a labelled virus was added ($\sim 1\%$ by volume, 1×10^4 particles per cell). Aliquots of cells were incubated at 34.5 °C, washed twice with cold medium and counted. Attachment of labelled virus is expressed in % of total added. Preparations of virus were usually dialysed against the MEM medium used for the cell suspension (adenovirus was in a buffer containing 10% glycerol, 0.001 M MgCl_2 , and 0.02 M Tris-HCl, pH 7.8; this was also used with 'mock' treated cells. Adenovirus fibre protein (2 mg ml^{-1}) was purified by chromatography on DEAE cellulose, hydroxylapatite and Sepharose 6B¹¹. The method of preparing, handling and counting the radioactivity of the cells has already been described³. 'Rhino-HeLa cells' were used in panels c, f, and i.

(Fig. 1), but 60 min at 22 °C or no pre-incubation but using a larger excess of unlabelled particles, gave compatible results (not shown). Excess unlabelled human rhinovirus type 2 (HRV-2) and type 14 (HRV-14), Coxsackie virus type A21 (CA21) and type B3 (CB3), poliovirus type 2 (P2), and adenovirus type 2 (Ad2) were employed for saturation of cellular receptors. These and other viruses were prepared and purified by methods already reported^{2,3,9,10}. The concentration of picornaviruses¹⁰ or adenoviruses² was estimated in particles per ml by ultraviolet adsorption. Pools of purified virus contained 1×10^{13} – 3×10^{13} particles per ml, and when labelled, 0.6×10^6 – 6×10^6 c.p.m. per ml.

Two lines of suspension-grown HeLa cells were used. The human rhinoviruses and Coxsackie virus A21 appeared to attach significantly more rapidly to one of these, 'rhino-HeLa-cells' than to the other, but the two lines otherwise showed no qualitative differences in competition experiments.

Efficient attachment of radioactive virus to cells required warming of the mixture. The rate of attachment varied from one type of virus to another and from experiment to experiment depending upon the cell line employed. Homologous competition for receptors was tested and detected in every experiment using excess virus or antigen.

Figure 1 illustrates representative results of experiments employing excess CB3, Ad2, or Ad2-fibre. As expected, attachment of ¹⁴C-labelled CB3 was inhibited by excess homologous virus (Fig. 1a), but unexpectedly, attachment of CB3 was also inhibited by excess Ad2 (Fig. 1b) or Ad2-fibre (Fig. 1c). In a reciprocal way, attachment of ³⁵S-Ad2 was blocked by excess CB3 (Fig. 1d) as well as by excess Ad2 particles or fibre protein (Fig. 1e and f). Thus, CB3 and Ad2 clearly share HeLa cell receptors.

Excess unlabelled CB3 did not inhibit attachment of labelled HRV-2, HRV-14, P2 (not shown) or labelled CA21 (Fig. 1g). A different picture emerged with unlabelled Ad2 which produced a clear twofold reduction in the rate of attachment of labelled HRV-2 (Fig. 1h). This was not

reciprocal, however, and excess HRV-2 had no effect on attachment of labelled Ad2 (not shown). Treatment of cells with the relatively small (molecular weight 200,000 (ref. 11)) Ad2 fibre protein had no effect on HRV-2 attachment (Fig. 1i), indicating that the large bulky Ad2 particles on one site may interfere with attachment of HRV-2 to a separate site by steric hindrances. Alternatively, adenovirus may induce cross linking of components of the cell surface, which may lead to alteration or internalisation of the receptors for HRV-2. HRV-2 and Ad2 may thus recognise either separate determinants on the same macromolecule or determinants on adjacent macromolecules. It would be interesting to determine if isolated purified receptors for Ad2 will interact with HRV-2 particles as well. Cells treated with excess Ad2 also showed a barely detectable (~10%) reduction in attachment of HRV-14, CA21, and P2 (not shown).

Figure 2 shows some of the effects of treating cells with excess CA21 or excess HRV-14 on the attachment of other viruses. Excess CA21 is able to inhibit attachment of both labelled homologous virus (Fig. 2a) and also labelled HRV-14 (Fig. 2c). Competition for receptors by excess HRV-14 was more difficult to demonstrate, probably because HRV-14 attaches slowly to HeLa cells³. Thus cells treated with excess HRV-14 attached significant amounts of CA21 or HRV-3 (Fig. 2b and f), but there was clear evidence for interference. Similar data have been found for the attachment of HRV-41 (ref. 3) and some other labelled rhinoviruses (HRV-5, 15, 39, 51) to cells treated with excess HRV-14, and they probably all share the same receptor with the poorly attaching HRV-14. It is interesting to note that although the B Coxsackie viruses also probably share one receptor, the rates of attachment differ widely and show entirely different pH optima¹². Neither CA21 nor HRV-14 were able to inhibit attachment of HRV-2, P2, CB3, or Ad2 (not shown, see also ref. 3 and Fig. 2e).

Our results are summarised in Table 1. There are four receptor families. The first family contains HRV-2,

Fig. 2 Attachment of labelled virus particles to untreated cells (●) or to cells treated with excess unlabelled CA21 or HRV-14 (○). Attachment of ¹⁴C-CA21 was inhibited by excess CA21 (a) or excess HRV-14 (b). Attachment of ¹⁴C-HRV-14 was inhibited by excess CA21 (c) or HRV-14 (d). ³²P-P2 was not inhibited by excess CA21 (e); HRV-3 was inhibited by excess HRV-14 (f). The method used is described in the legend to Fig. 1. 'Rhino-HeLa cells' were used in panels b, d, e, and f.

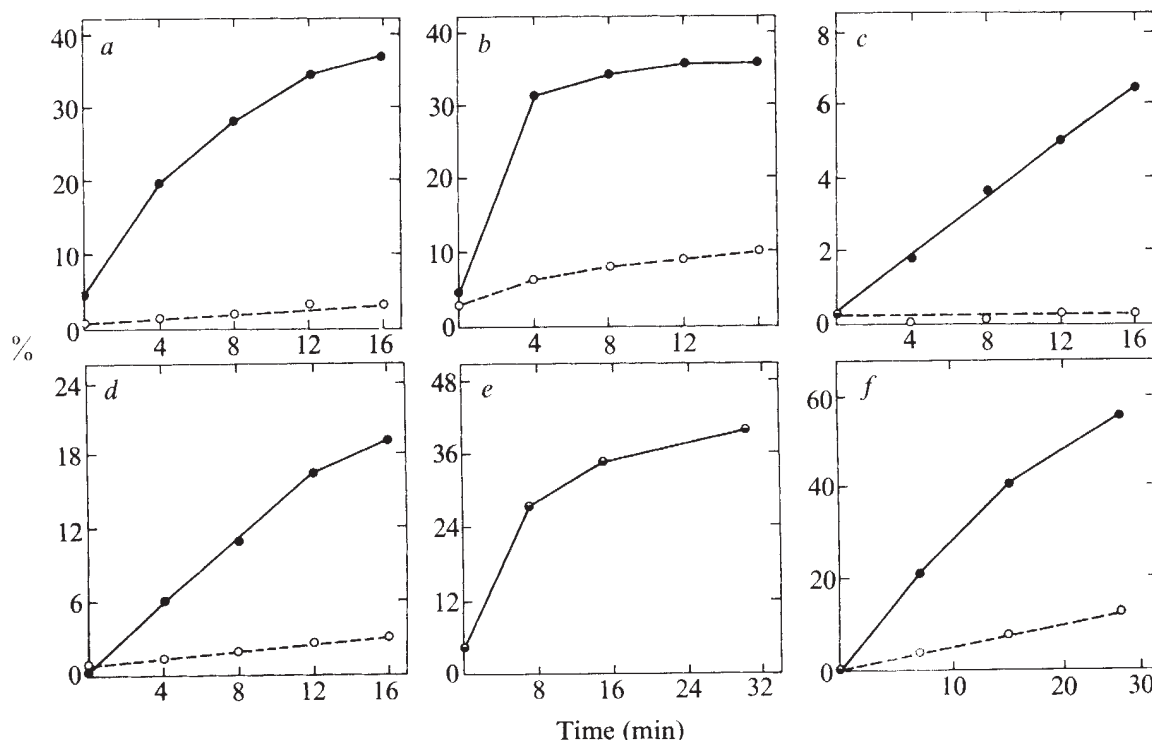


Table 1 Virus receptor families detected by competition for attachment to HeLa cells*

In presence of excess	HRV-2	HRV-14	CA21	P2	CB3	Ad2
HRV-2†	+	—	—	—	—	—
HRV-14‡	—	+	+	—	—	—
CA21	—	+	+	—	—	—
P2	—	—	—	+	—	—
CB3	—	—	—	—	+	+
Ad2	±§	(-)	(-)	(-)	+\$	+

*Strains of virus used: HRV-2(HGP), HRV-14(1059), CA21(48654), P2(P217-Ch2ab), CB3(Nancy), Ad2(Adenoid 6), HRV-1A(2060), HRV-1B(B-632), HRV-3(FEB), HRV-5(Norman), HRV-15(1734), HRV-39(209), HRV-41(56110), HRV-51(F01-4081).

†In addition to competition (+) with attachment of homologous virus, excess HRV-2 also inhibited attachment of HRV-1A and HRV-1B (ref. 3, and Lonberg-Holm, unpublished data). Excess HRV-14 did not inhibit attachment of HRV-1A or HRV-1B.

‡In addition to the homologous and reciprocal inhibitions (+) indicated, excess HRV-14 also inhibited attachment of HRV-3, -5, -15, -39, -41, and -51. Excess HRV-2 did not inhibit these viruses (ref. 3, and Lonberg-Holm, unpublished data).

§Adenovirus fibre protein and intact Ad2 blocked attachment of CB3. Intact Ad2, but not excess fibre, partially inhibited attachment of HRV-2.

||Attachment of HRV-14, CA21 and P2 was inhibited by ~10% following treatment of cells with excess Ad2.

HRV-1A and HRV-1B. The second family contains HRV-14 and CA21. Other experiments, with excess HRV-14, indicate that HRV-3, -5, -15, -39, -41 and -51 belong in this family (unpublished data, see also Fig. 2f and ref. 3). The third family contains poliovirus type 2, and therefore all three serotypes of poliovirus^{6,12}. The fourth family contains Coxsackie virus B3 and thus probably all or most of the other B Coxsackie virus^{7,12}. Ad2 and probably at least several other adenoviruses are in this family³.

It is possible that receptor specificity influences virus tropism in tissues and organs, and thereby contributes to patterns of viral pathogenesis^{13,14}. The finding that Coxsackie virus A21 ('Coe virus') shares receptors with a large number of human rhinoviruses may be related to its ability to produce cold-like symptoms¹⁵. Both B Coxsackie viruses^{1,16} and adenoviruses¹⁷ produce persistent infections in HeLa cells cultured in the presence of human serum. It will be important to determine if receptors for CB3 and Ad2, or CA21 and HRV-14 are covariant in different tissues or different host species.

Future work should attempt to determine the physiological and biochemical identity of the specific virus receptors, this could be rewarding because virus receptors have been used as markers in cytogenetic studies^{4,18}.

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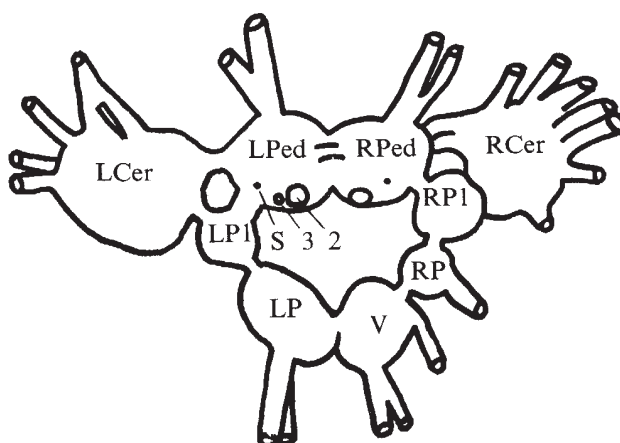
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Two kinds of cholinergic receptors on the membrane of the completely isolated identified *Planorbarius corneus* neurone

WE have found that an identified neurone of the *Planorbarius corneus* left pedal ganglion (LPed-2 neurone, Fig. 1) responds with depolarisation to nicotinomimetics and with hyperpolarisation to some muscarinomimetics. The LPed-2 neurone of *P. corneus* contains dopamine¹ and is the dopaminergic interneurone sending depolarising and hyperpolarising impulses to some neurones of the visceral and the left parietal ganglion². Acetylcholine (ACh) alone produces depolarisation like a nicotinomimetic; in the presence of tubocurarine, ACh hyperpolarised the cell membrane like a muscarinomimetic. The suggestion has been made that two kinds of cholinergic receptors (ChR) exist on the membrane of the LPed-2 neurone, differing in both the pharmacological properties and the ionic permeability changes they control³. Two different types of ChR have been described on the membrane of some identified *Aplysia* neurones^{4,5}. Experiments³ performed on the LPed-2 neurone in the ganglionic ring where it receives synaptic inputs from various interneurons which also can respond to ACh do not exclude the possibility that the two kinds of responses are attributable to the interference of some interneurons masking the actual effect of ACh on the LPed-2 neurone.

To resolve this problem we performed experiments on completely isolated LPed-2 and LPed-3 neurones (Fig. 1). The method described by Kostenko⁶ was used. The ganglionic

Fig. 1 Diagram of the dorsal surface of *P. corneus* circumoesophageal nerve ring. Cerebro-cerebral connective is cut to expose the pedal ganglia. Ganglia: LPed, left pedal; LCer, left cerebral; LPl, left pleural; LP, left parietal; V, visceral; RP, right parietal; RPl, right pleural; RCer, right cerebral; RPed, right pedal. LPed-2 (2) and LPed-3 (3) neurones can be easily identified: LPed-2 is the biggest of the left pedal ganglion¹; LPed-3 can be identified by its position between LPed-2 and statocyst (S) and by the characteristic red colour of the surrounding glial cells.



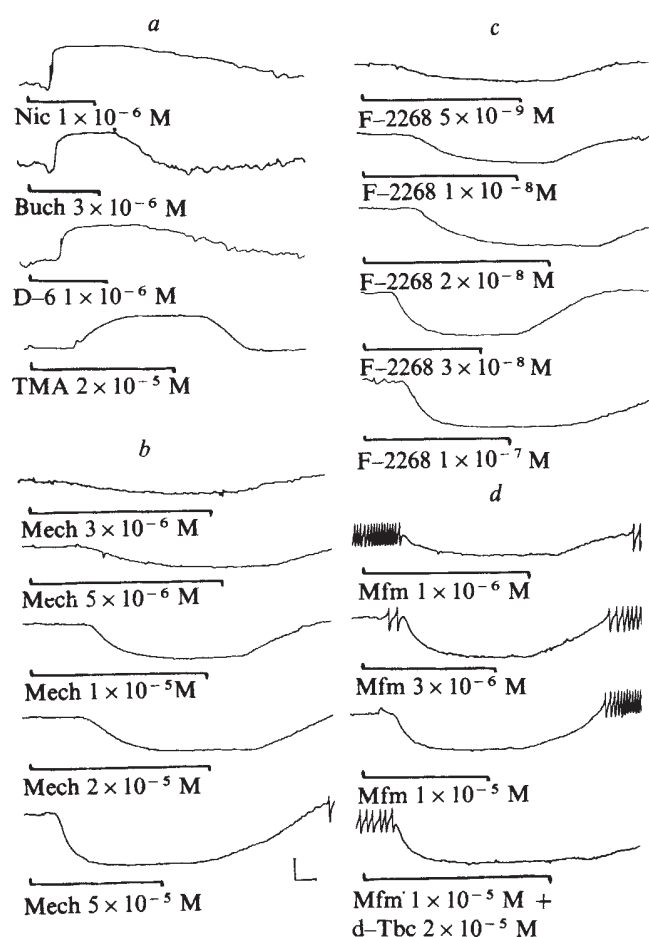
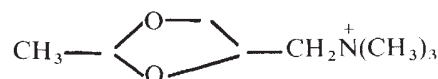


Fig. 2 Effects of nicotinomimetics and muscarinomimetics on the completely isolated LPed-3 neurone. All records were obtained in the same experiment. The resting transmembrane potential was equal to -60 mV. Drugs were added to the perfusion fluid at the upward arrow and washed out at the downward arrow. Calibration 10 s, 10 mV here and in Fig. 3 refers to de- and hyperpolarisation but not to the spikes. *a*, Depolarising responses to nicotinomimetics. Approximately equally effective concentrations were chosen. Nic, nicotine; BuCh, butyrylcholine; D-6, suberyldicholine¹⁷ $(\text{CH}_3)_3\text{N}-\text{CH}_2\text{CH}_2-\text{O}-\text{CO}-(\text{CH}_2)_6-\text{CO}-\text{O}-\text{CH}_2\text{CH}_2-\text{N}(\text{CH}_3)_3$; TMA, tetramethylammonium. *b-d*, Hyperpolarising responses to muscarinomimetics. *d*, Methylfurmethide (Mfm); note that at Mfm concentrations of 10^{-5} M hyperpolarisation is greater in the presence of tubocurarine showing that Mfm activates at this concentration both kinds of ChR hyperpolarising response being partly masked by simultaneous depolarisation. *b*, Acetyl- β -methylcholine (Mech). *c*, dioxolan F-2268.



Note that F-2268 is the most potent: its equieffective hyperpolarising doses are three orders of magnitude smaller than those of Mech or Mfm.

4 mM CaCl_2 , 8 mM MgCl_2 , Tris-buffer 0.25 g l^{-1} , $\text{HCl} \leq \text{pH } 7.5$) and impaled with a microelectrode filled with 2.5 M KCl. Membrane potential was recorded using the standard intracellular technique⁷. Drugs were added to the perfusion fluid.

After isolation the LPed-2 neurone retains the rhythmic spontaneous activity which it characteristically displays in the ganglion. In the ganglion the LPed-3 neurone showed non-rhythmic discharges or permanent oscillations of transmembrane potential. After isolation it usually became silent or discharged rhythmically.

We found that LPed-2 and LPed-3 neurones in the isolated state are depolarised by nicotinomimetics (Fig. 2*a*) and hyperpolarised by muscarinomimetics (Fig. 2*b*, *c*, and *d*); this means that the isolated neurones respond in the same way as when they are *in situ* in the brain. The response to ACh is more complicated and depends on the concentration used (Fig. 3). Small ACh concentrations (5×10^{-8} M– 2×10^{-7} M) caused hyperpolarisation. With greater concentration the cell begins to repolarise in the presence of ACh; after washing out the hyperpolarisation reappears. This response suggests that ACh

circumoesophageal ring was treated with 0.3% Pronase solution for 30–40 min, then the sheaths were removed and single identified neurones were isolated using electrolytically sharpened tungsten needles. The isolated neurone was placed in a chamber (1.5 ml) perfused with saline solution (50 mM NaCl, 1.6 mM KCl

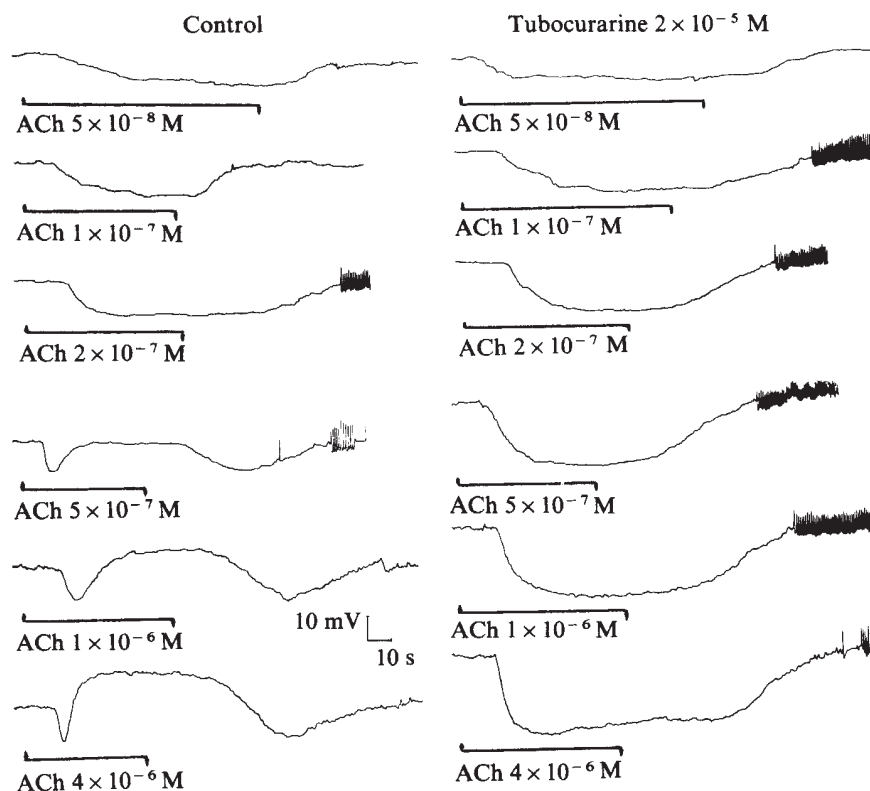
Table 1 The ionic dependence of depolarising and hyperpolarising responses of the isolated LPed-3 neurone to cholinomimetics

Drug and concentration (mol)	a. Changes in the external chloride and sodium concentration									
	Responses (mV)									
	at different [Cl] ⁻ concentrations (mM)									
	[Cl] ⁻ 75*	[Cl] ⁻ 38	[SO ₄] ²⁻ 38	[Cl] ⁻ 75*	[Cl] ⁻ 38	[C ₂ H ₅ COO] ⁻ 38	[Cl] ⁻ 75*	[Na] ⁺ 50*	[Na] ⁺ 25	[Li] ⁺ 25
ACh (1×10^{-6})	+11					+28	+11			
ACh (2×10^{-6})	+14					+20	+15			
ACh (1×10^{-5})	+7					+17				
ACh (5×10^{-7})	+11	+20		+10						
Nic (1×10^{-6})	+11	+16								
D-6† (1×10^{-6})	+11	+18				+19	+11	+11	+12	+11
F-2268 (1×10^{-6})	-13	-12								
F-2268 concentrations (Mol)	b. Changes in the external potassium concentration									
	Response (mV)									
	External potassium concentrations (mM)									
	1.6*	0.8		1.6*	1.6*		3.2		1.6*	
1×10^{-8}	-4	-9								
5×10^{-8}	-15	-23		-21						
1×10^{-7}	-21	-28								
5×10^{-8}	-10	-20		-13						
1×10^{-7}							-19		-8	
3×10^{-7}							-21		-12	
2×10^{-8}	-4	-11		-4						
5×10^{-8}	-7	-17		-8						
2×10^{-7}							-15		-3	

*Normal saline

†D-6 (suberyldicholine)¹⁷ is a nicotinomimetic agent deprived of any muscarinic properties.

Fig. 3 Effect of tubocurarine on the responses of the completely isolated LPed-3 neurone to ACh. All records were obtained in the same experiment. Note that with small ACh concentrations there is no difference between control responses (left column) and those in the presence of tubocurarine (right column); probably N-ChR are not excited by these ACh concentrations. At 2×10^{-7} M hyperpolarising response is greater in the presence of tubocurarine. Higher ACh concentrations caused a two-component potential change in the control but pure hyperpolarisation in the presence of tubocurarine (with the exception of response to ACh (4×10^{-6} M) where a slight repolarisation can be seen, suggesting that the tubocurarine concentration (2×10^{-5} M) is not high enough to protect the nicotinic ChR against ACh concentration as high as 4×10^{-6} M. The resting potential was equal to 43 mV. Tubocurarine (2×10^{-5} M) was added to the perfusion fluid 2 min before the addition (upward arrow) of the solution containing both ACh and tubocurarine. Calibration 10 mV, 10 s.



activates two kinds of ChR, with those mediating hyperpolarisation being sensitive to lower ACh concentration. Nicotinic ChR (N-ChR) of *P. corneus* and *Limnaea stagnalis* neurones are blocked by tubocurarine^{8,9} as are those of *Aplysia* neurones¹⁰. We observed that in the presence of tubocurarine ACh caused pure hyperpolarisation, as do muscarinomimetics (Fig. 3). These results indicate that two kinds of ChR coexist on the membrane of the completely isolated neurones, LPed-2 and LPed-3, with the isolated cells much more sensitive to ACh and cholinomimetics (especially muscarinomimetics) than non-isolated neurones.

Of the two types of ChR found on the membranes of the isolated neurones those mediating depolarisation are similar to N-ChR in vertebrates: they are excited by all nicotinomimetics and are blocked by tubocurarine. Receptors mediating hyperpolarisation seem to resemble the muscarinic ChR (M-ChR) of vertebrates less closely. We have studied the hyperpolarising potency of a series of drugs known to excite M-ChR: dioxolan F-2268 (ref. 11); methylfurmethide, acetyl- β -methylcholine; oxotremorine; arecoline; carbacholine; pentytrimethylammonium. All of those drugs (with the exception of oxotremorine) caused hyperpolarisation. The most potent proved to be F-2268 ($EC_{50} = 2 \times 10^{-8}$ M) which is very active on vertebrate M-ChR (refs 11 and 12). Other muscurinomimetics—even those known to have a high potency on vertebrate M-ChR (such as methylfurmethide and oxotremorine)—were far less potent than F-2268 in producing mollusc neurone hyperpolarisation. For example, methylfurmethide proved to be 350 times as weak as F-2268. Oxotremorine produced no hyperpolarisation even in high concentration. It seems, therefore, that mollusc neurone ChRs mediating hyperpolarisation differ in their chemical sensitivity from vertebrate M-ChR.

The difference becomes even more evident when studying the potency of typical muscarinolytics. Neither atropine nor scopolamine can prevent the hyperpolarisation caused by ACh or by muscarinomimetics. The alkylating agent benzilycholine mustard (A-5 ref. 13), known to block selectively and irreversibly vertebrate M-ChR (refs 14 and 15), was found to block

neurone hyperpolarisation selectively and irreversibly as well, without changing the depolarising (nicotinic) response. The concentration needed (2×10^{-4} M for 15 min) was, however, five orders of magnitude greater than that needed to block M-ChR in the guinea pig ileum. Like the slow hyperpolarisation of *Aplysia* neurones⁵, the hyperpolarisation of LPed-2 and LPed-3 neurones can be prevented by tetraethylammonium (5×10^{-5} M).

The study of the ionic basis of the depolarising and hyperpolarising responses are in progress; Table 1 shows the preliminary results. We have found (Table 1) that the replacement of 38 mM (one half) of the chloride ions in physiological solution by 38 mM of sulphate or propionate ions increases the depolarising responses to ACh, nicotine and the nicotinomimetic agent suberyldicholine (D-6), (ref. 16). The replacement of one half (25 mM) of sodium chloride in physiological solution by an equal concentration of lithium chloride did not change the depolarising response to D-6. These results suggest a chloride dependence of the depolarising response to ACh and to nicotinomimetics. As we used microelectrodes filled with KCl it can be concluded that the depolarising response to ACh and nicotinomimetics is attributable to the increase of the intracellular chloride concentration (as a result of the diffusion of chloride from the microelectrode) which in turn leads to the change in the chloride equilibrium potential and finally to the change in the sign of response. To check this possibility the microelectrodes filled with potassium citrate (1 M), K_2SO_4 (0.5 M) and Na_2SO_4 (1 M) were used in several experiments. Nicotinomimetic agents still caused depolarisation with these microelectrodes and there was a depolarising phase in the response to ACh.

No change in the hyperpolarising effect of F-2268 was observed when the chloride concentration was halved (Table 1). Halving (doubling) the potassium concentration increased (decreased) the hyperpolarising response to F-2268 (Table 1b). Thus this response seems to be potassium dependent.

Two kinds of ChR found on completely isolated *P. corneus* neurones seem to closely resemble those on the neurones of

the *Aplysia* pleural ganglion⁶ in both pharmacology and ionic dependence.

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Fluorescence changes during electrical activity in frog muscle stained with merocyanine

NERVE membranes stained with various fluorescent dyes show changes in fluorescence intensity concomitant with changes in transmembrane potential^{1–4}. Cohen *et al.*⁴ described a number of dyes that exhibit changes in fluorescence emission which vary linearly with transmembrane potential. Two of these have been used to investigate the excitation–contraction process in skeletal muscle^{5–7}. Whole muscles and single fibres stained with Nile Blue A were reported to give fluorescence intensity changes on stimulation arising from the membranes of the sarcoplasmic reticulum^{5,6}. Muscles and fibres stained with Merocyanine 540 on the other hand, give very early changes in fluorescence intensity when stimulated electrically^{6,7}. Landowne⁷ proposed that since the merocyanine signal disappeared after detubulation in snake costocutaneous muscles, the fluorescence change must arise from the T-system membranes.

We describe here experiments in which the extrinsic fluorescence signal is compared with the membrane potential in bundles of frog skeletal muscle fibres stained with Merocyanine 540. Our results partially agree with Landowne's conclusion that this dye monitors electrical activity in the T-system membranes; they do indicate, however, that the surface membrane also contributes to the fluorescence signal. The proportion of the contributions from both membrane systems can be roughly estimated as 9:1 (T-system–surface membrane) but important assumptions underlying this value must be investigated in detail.

Bundles of 40–200 fibres from the sartorius and the semitendinosus muscles of the Chilean frog, *Calyptocephaleya gayi* were used instead of single fibres to extend the life of the preparation exposed to the toxic effects produced by the merocyanine dye in the presence of light and oxygen (see below and ref. 4). They were dissected and mounted horizontally in the narrow trough of a lucite chamber. The trough was separated into three compartments with Vaseline. The central compartment (4 mm wide) had one

of the walls and the bottom made of glass to allow light transmission. The other wall was a platinised Pt plate used to pass current through the muscle fibres to the lateral compartments. The bundles were stimulated in two different ways; mode A, by passing current between the central compartment and the lateral ones to give simultaneous polarisation of the fibres in the central compartment; and mode B, by passing current between two Pt plates in one of the lateral compartments to propagate action potentials travelling to the central compartment. The bundle was kept in place by connecting one tendon to a strain gauge transducer and the other to forceps. The chamber was placed on an optical bench so that the light coming from a tungsten–halogen lamp could be filtered and focused on the bundle in the central compartment of the chamber. At 90° with respect to the illuminating beam, the light was filtered and collected by a photodiode connected to the recording apparatus (for details see legend of Fig. 1 and ref. 5). As fibres stained with Merocyanine 540 were damaged by exposure to light in the presence of oxygen in the bathing medium, the illumination periods were minimised and nitrogen was bubbled in the bathing solution between experimental runs. Nevertheless, after several periods of illumination, action potentials became wider and the fibres eventually lost their excitability.

Figure 1 shows the relationship between the fluorescence signal (trace a) and tension development (trace b) in a bundle of about 40 sartorius fibres stimulated by a single-current pulse in mode B. The bundle had been exposed to the staining solution for 45 min and then transferred to Ringer's solution for recording. Figure 1a shows a transient fluorescence signal followed by a movement artefact when tension starts to develop (trace b). To eliminate this artefact, and to observe the recovery phase of the early

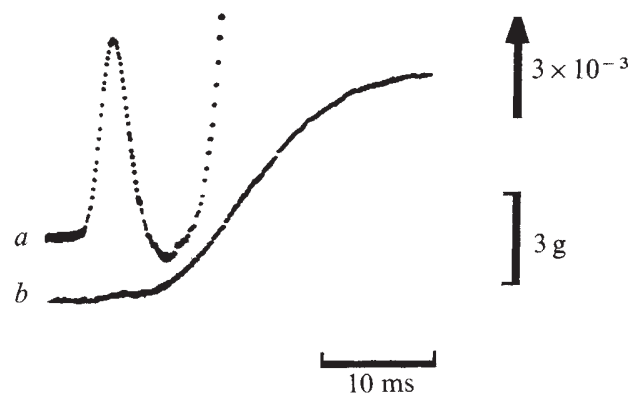


Fig. 1 Merocyanine fluorescence signal (a) and tension (b). The change in fluorescence is expressed as the ratio of change in light intensity to resting fluorescence light intensity. An increment in fluorescence intensity is indicated by the arrow. Temperature 21 °C. In all experiments a Baird-Atomic interference filter (35-15-16) with a central wavelength of 548.6 nm was used as a primary filter to render the incident light quasi-monochromatic. The light from the bundle was filtered by a barrier filter (Schott RG-610) which absorbed wavelengths shorter than 610 nm. This was used to filter the light emitted at 90° with respect to the source and was placed beneath the bottom of the central compartment of the chamber. Below the secondary filter, the light was collected by a photodiode (PV-444, E.G. & G.) followed by a current–voltage converter. The electrical signals from the photodiode were filtered by a bandpass filter (time constant 0.2 ms), amplified by a Tektronix 3A3 amplifier and then fed with either the membrane potential from a microelectrode amplifier or the tension signal from the transducer into a digital machine which will be described elsewhere. This machine enabled us to average the signal and to record the output using a standard high-fidelity tape recorder or chart recorder. The Ringer's solution contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 2.15 mM Na₂HPO₄ and 0.85 mM NaH₂PO₄, pH = 7.1 (ref. 8). The staining solution was Ringer's plus 0.05 mg ml⁻¹ Merocyanine 540 (Eastman Kodak), Pluronic F-127 0.02% (BASF Wyandotte) and 1% ethanol.

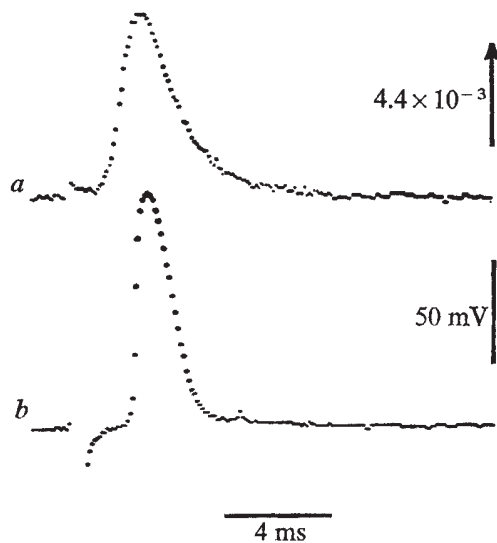
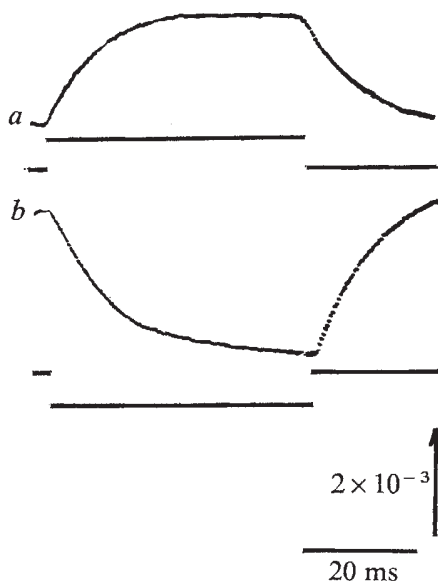


Fig. 2 Fluorescence signal and action potential. *a*, A single sweep displaying the change in fluorescence intensity on stimulation of a bundle of more than 100 fibres from the semitendinosus muscle. *b*, Action potential recorded by a microelectrode impaled in a fibre with a resting potential of -86 mV in the centre of the illuminated region of the central compartment. Both records were taken simultaneously for a single stimulus of 0.2-ms duration in the depolarising direction. Capacitive pick-up by the microelectrode distorted the membrane potential record at short times after the stimulus pulse was given. The bundle was stretched to 1.4 times its slack length and was immersed in 345 mM NaCl hypertonic Ringer's solution (see text). The tension record was flat. Temperature 22°C .

signal it was necessary to block development of tension. This was achieved by stretching the bundles to between 1.3 and 1.5 times their slack lengths and immersing them in hypertonic solutions with 230 mM NaCl added to Ringer's solution⁵. Figure 2 shows the results of an experiment in those conditions. The bundle was stimulated with a 0.2-ms

Fig. 3 Fluorescence intensity changes with prolonged stimulation. *a*, Fluorescence intensity change during a depolarising current pulse after 10^{-6} M TTX was added to the Ringer's solution. *b*, The resulting fluorescence signal during a hyperpolarising pulse of equal amplitude. Both records are the average of 20 sweeps. The time courses of the stimulus pulses are shown below each trace. The bundle was stretched to about 1.5 times its slack length and it was immersed in hypertonic Ringer's to prevent movement during depolarisation. Temperature 20°C .



depolarising current pulse in mode A. The complete time course of the transient fluorescence signal is displayed, without the movement artefact, in trace *a*. Trace *b* is the simultaneous record of an action potential obtained by impaling a single fibre from the bundle with a microelectrode positioned in the centre of the illuminated region. The rising phase of the action potential ($t_{1/2}=0.5$ ms) is steeper than the rising phase of the fluorescence signal ($t_{1/2}=1$ ms) and the recovery phase of the latter ($t_{1/2}=1.2$ ms) is slower than the repolarisation of the action potential ($t_{1/2}=0.95$ ms). One reason why records 2*a* and *b* are different could be that the fluorescence signal is recorded simultaneously from a population of fibres of the bundle. The non-synchronous activation, due to different thresholds among fibres of the bundle, would be seen as an apparently slower signal. This possibility was tested by impaling several fibres in the illuminated region and different delays were found between the peak of the action potential and the peak of the fluorescence signal. In the experiment shown in Fig. 2, the impalement of five fibres showed that the peak of the fluorescence signal preceded, or was delayed with respect to the peak of the action potential within a range of 0.85 ms. This range would explain the differences on the rising phase half times between the fluorescence signal and the action potential. This argument, however, is less convincing in explaining the differences in the falling phase half times because the repolarisation of the action potential is a slower process. An alternative, but not exclusive, explanation for the differences between the action potential and the light signal time courses would be that the latter is monitoring the slower potential changes of the T-system membranes⁹. In fact, the merocyanine fluorescence signal recorded from a single fibre by Oetliker *et al.*⁸ has a similar time course to that shown in Fig. 2*a*.

Figure 3 shows the fluorescence signals recorded in a bundle after addition of 10^{-6} M tetrodotoxin (TTX) to the external solution, when 45-ms hyperpolarising (Fig. 3*a*) and depolarising (Fig. 3*b*) pulses were applied in mode A. The characteristic 'creep', produced by the anomalous rectifier, is observed in Fig. 3*b*. The time constant of the membrane as monitored by the dye was calculated from these and three more experiments to be 17.9 ± 1.22 (s.e.) ms ($n=7$). This value is close to those measured with microelectrodes^{12,13}. If most of the fluorescence signal is coming from the T-system membranes, the optical time constant should be slightly longer than the electrical one. This difference, however, might be within experimental error in our measurements.

To obtain more information about the membrane systems monitored by the merocyanine dye, we carried out a series of detubulation experiments using the procedure described by Eisenberg *et al.*¹⁰. In this method the bundle is transferred from the glycerol-containing solution to Ringer's containing 5 mM Ca and 5 mM Mg which maintains the resting potential at around -82 mV (ref. 15). Figure 4*a* corresponds to a propagated fluorescence signal before detubulation of a bundle of less than 60 fibres immersed in hypertonic solution and stimulated in mode B. The bundle was stimulated 19 mm away from the illuminated region and the propagated signal was detected about 11.3 ms after the stimulus pulse. The speed of propagation of the signal can be roughly calculated to be 168 cm s^{-1} , which agrees with previous measurements of action potentials speed¹⁴. After detubulation, the fluorescence signal changed (Fig. 4*b*). The amplitude of the signal is reduced by a factor of ten and the propagation velocity increased to 229 cm s^{-1} , 36% faster than before detubulation. The decay phase of the light signal in the detubulated bundle (Fig. 4*b*) is faster than that shown in Fig. 4*a*.

The fluorescence signal was used in two experiments to measure the time constant of the membrane, obtained with long pulses applied to TTX-treated bundles. The average

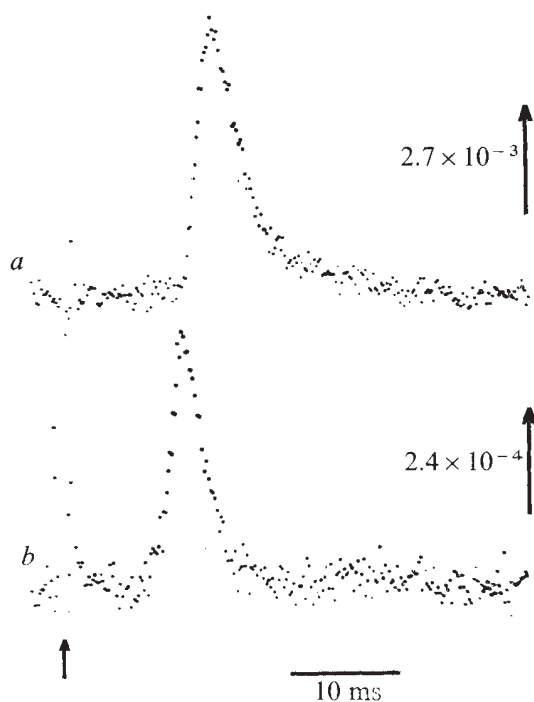


Fig. 4 Propagated fluorescence signals before and after detubulation. *a* and *b* were obtained from the same bundle of 60 semitendinosus fibres in 345 NaCl hypertonic Ringer's solution and stretched to about 1.5 times its slack length. *a*, A single sweep before detubulation; *b*, average of 40 sweeps after detubulation. The arrow indicates the time the stimulus pulse was applied. Pluronic 127 was not used in the staining solution. Temperature 20 °C.

value was 7.33 ± 0.79 (s.e.) ms ($n=6$), significantly faster than in intact bundles. No significant 'creep' was observed during hyperpolarising pulses. These results demonstrate that detubulation has a similar effect on the fluorescence signal and the membrane potential recorded with microelectrodes. The amplitude of the fluorescence signal, however, is reduced by the glycerol treatment, but the action potential amplitude is virtually unaffected¹⁸. If we assume that the magnitude of the signal is proportional to the amount of membrane stained, we estimated that ~10% of the total membrane is not affected by detubulation. In other experiments this value ranged from 4% to 20%. It could be argued that the 90% decrease of the light signal is produced by damage during detubulation. Apart from evidence that the glycerol treatment, using raised Ca and Mg concentrations, gives a better survival of the fibres^{10,15}, there are several observations which make this explanation unlikely. First, the increased speed of the falling phase of the fluorescence signal after detubulation is consistent with the idea that Merocyanine 540 monitors the electrical activity of both surface and T-system membranes in intact fibres, but predominantly surface membrane in detubulated fibres. Second, the faster time constant of the light signal in response to long pulses in TTX-treated fibres after detubulation is also compatible with the loss of T-system membrane capacity—which is predominant in intact fibres and there produces a longer time constant for the light signal. Finally, in the experiments performed in normal Ringer's, tension was reduced by detubulation to a lower proportion (0.4–2.2%) than the light signal amplitude (4–20%), indicating that most of the remaining fluorescence signal came from fibres that were inactivated mechanically; this in turn, indicates that the signal originated partially in the surface membrane. More detubulation experiments will be required to study in detail the relative contribution of the surface and tubular membranes and the qualitative changes in the

time course of the fluorescence signal before and after detubulation.

Merocyanine 540 very probably does not penetrate to the interior of the fibres because a larger and slower signal would be expected from the sarcoplasmic reticulum, like that seen with the lipid-soluble dye Nile Blue A^{5,6}. Also, recent experiments in squid axon microinjected with Merocyanine 540 suggest that the dye, with its fixed negative charge, does not cross the neuronal membrane rapidly (B. M. Salzberg, personal communication).

The results presented here may prove useful for those investigations in which monitoring membrane potentials from a population of muscle fibres is required. They complement information obtained with other dyes^{5,6} that monitor membrane potential changes in the sarcoplasmic reticulum. Using Merocyanine 540 and Nile Blue A it is now possible to study separately the potential changes across different membrane systems, some of them inaccessible to microelectrodes and intimately involved in the chain of events of the excitation–contraction coupling in skeletal muscle.

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Effect of temperature and pressure on polymerisation equilibrium of neuronal microtubules

MICROTUBULES are fibres, sometimes many micrometres long, present in all eukaryotic cells, formed by the reversible polymerisation of the protein tubulin. These fibres not only have a structural role, but the process of their assembly and disassembly itself controls several cell functions¹.

We have investigated the effect of temperature and pressure on this process, and show here that their main influence is on the nucleation equilibrium. With this reanalysis of the data, we make some speculations about some of the molecular processes involved.

No detailed molecular model of the reaction is yet available. Depolymerisation, either by adding calcium or cooling to 4 °C, gives a mixture of a 6S protomer (an $\alpha\beta$ -dimer of total molecular weight 120,000) and a 36S fraction². This 36S protein is a ring or spiral-like structure of ~23 protomers, formed in the presence of the 'τ factor'³. Copolymerisation of the 6S and 36S fractions leads to the formation of the microtubules.

Until now equilibrium data for the polymerisation have always been analysed in terms of a propagation equilibrium constant, the reciprocal of the critical concentration. In this way Gaskin *et al.*⁴ were able to calculate an apparent reaction enthalpy change for propagation of 21 kcalorie mol⁻¹, for temperatures < 25 °C, and the corresponding entropy change of 100 EU. At temperatures between 25 and 37 °C the enthalpy change is essentially zero and the entropy change is ~ 10 EU. The effect of pressure has been analysed^{5,6}, using the same assumption, at a single protein concentration. It was found that propagation is accompanied with a volume change of 90 ml mol⁻¹.

Essential in all these analyses is the assumption that the protomer concentration remains constant from the critical protein concentration on. This is, however, true only in the limits of very high protein concentration, and not in the range usually studied, as shown by ultracentrifugation⁷ and by the data of Fig. 1. Here the change of turbidity at 350 nm is plotted as a function of total protein concentration, at different temperatures. The reversibility was checked in separate experiments. The data clearly show that the protomer concentration is not constant, both because the concentration range studied is rather low, and because an additional equilibrium, the nucleation equilibrium, determines the protomer distribution.

The effect of pressure was analysed at 35 and 15 °C, by measuring light scattered at 90 °C, as described earlier⁸. Experiments were done in conditions of complete reversibility which was maintained up to 1,000 atm at 35 °C. The increase in pH of the MES buffer with increasing pressure was very small and so was neglected. The results are given in Fig. 2, which shows that the nucleation equilibrium is again important. The results of Fig. 2 also show that there is a link between the effect of pressure and temperature. At 15 °C, 500 atm is sufficient to produce complete dissociation, while at 35 °C only a small pressure effect is observed. A link between the effect of pressure and temperature is also found for a number of other proteins, such as flagellin⁹ and glutamate dehydrogenase¹⁰, although the effect is much less pronounced for these. O'Connor *et al.*¹¹ did not find a measurable pressure effect at 37 °C and 700 atm, and

Fig. 1 The effect of temperature on the turbidity of microtubules at different total concentrations. Rat brain tubulin was prepared according to Shelanski¹⁶. After the third polymerisation cycle it was dialysed against MES buffer at pH 6.4, with the following composition: 50 mM MES, 70 mM KCl, 1 mM EGTA and 0.5 mM MgCl₂. After dialysis GTP was added up to 1 mM. Protein concentration was determined with the method of Lowry¹⁷. The quality of the microtubules was checked by electron microscopy.

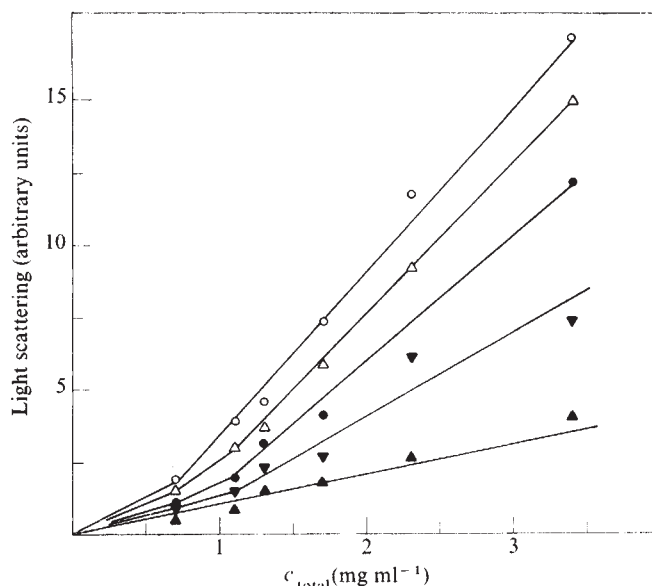
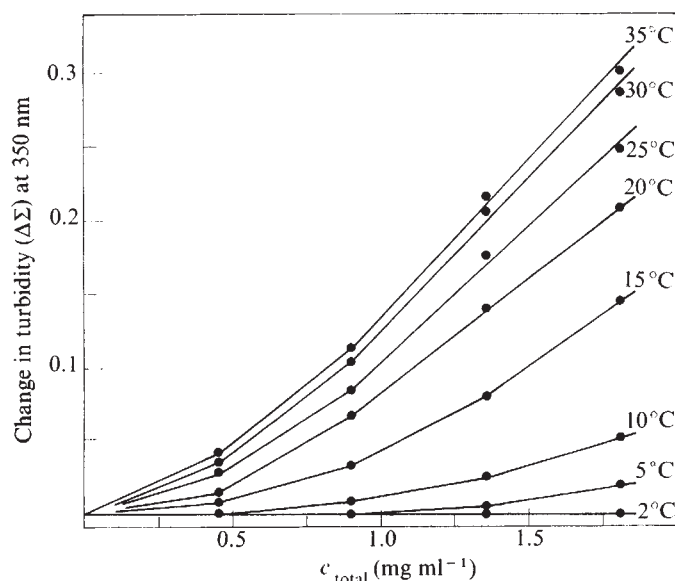


Fig. 2 The effect of pressure on light scattering by microtubules at different total concentrations. The same buffer as in Fig. 1 was used. The accuracy of the measurements is much less than in Fig. 1, because all the measurements had to be done on samples from different preparations. For the sake of clarity only a limited number of data are given. The measurements are corrected for window effects and lowering of the incident light intensity as a consequence of turbidity. ○, data at 1 atm at 35 °C; △, 500 atm at 35 °C. ●, ▽, ▲, for 1, 200, 500 atm respectively at 15 °C.

they claim that neurotubules are therefore different from other microtubules on the basis of a different pressure sensitivity, without taking into account the effect of the temperature. At 20 °C, where microtubules of non-neuronal origin are usually studied, neurotubules also show a large pressure dependence.

We have attempted to analyse these data quantitatively in terms of an equilibrium constant for nucleation and propagation. The system behaves completely reversibly in the presence of an excess of GTP, whose role is not, however, clear. The thermodynamic parameters obtained are therefore equilibrium parameters for the polymerisation coupled to GTP-hydrolysis, or activation parameters in the case of an irreversible consumption of GTP and the development of stationary states.

We assume that there is a nucleation step and that growth only occurs at the nuclei or tube ends (see ref. 13). (In fact Dentler *et al.*¹⁴ show that growth occurs only at one end of the tube.) The relationship between c_p , the number concentration of protomers present in polymers, and c_1 the protomer concentration is then:

$$c_p = Ac_1/(1 - Kc_1)^2 \quad (1)$$

Here K is the propagation equilibrium constant, which is assumed to be independent of the degree of polymerisation (i) for $i > n$, where n is the number of protomers involved in the nucleus. AK^{n-1} gives the nucleation equilibrium constant, with A a dimensionless equilibrium constant that corresponds to the destabilisation of the nucleus with respect to an n -mer in the middle of the polymer. This equation does not necessarily give the right distribution for the intermediate stages between protomers and nucleus. The contribution of these terms is, however, negligible. Equation (1) can then be rearranged

$$(c_1/c_p)^{1/2} = A^{-1/2}(1 - Kc_1) \quad (2)$$

and the data are analysed accordingly.

Gaskin *et al.*⁴ showed that light (wavelength λ) scattered by microtubule solutions is nearly independent of the length of the tubules which can be proved directly by sonication of the

tubules, and also by the dependence of the scattering on λ^{-3} . We found that this dependence is maintained down to 8 °C, while at 4 °C a λ^{-4} dependence was found. This indicates that light scattering is indeed a good means of measuring c_p even at the lowest degrees of polymerisation.

Assuming that the steepest slope in Fig. 1 corresponds to the specific turbidity of the polymers, as checked by ultracentrifugation, c_1 and c_p can easily be calculated. As in previous treatments^{4,6} c_1 is defined as the total number concentration of protomers, regardless of their distribution between 6S and 36S forms. Figure 3 shows that equation (2) fits the data well. The parameters A and K can be calculated simply and their logarithm plotted against T^{-1} , as shown in Fig. 4a. These data show that in the range of 25–10 °C the enthalpy and entropy changes are much larger for the parameter A than for the propagation equilibrium constant. In this case the theory predicts that at low temperatures, where the nucleus is destabilised, a small number of very long tubules will be present. This is confirmed both by the inverse third power dependence on the wavelength and by electron microscopy.

In the range of 35–30 °C smaller values for the thermodynamic parameters are found (see ref. 4). This change may be caused by a conformational transition of the protein, although the presence of a small amount of inactive protomer would cause a deviation in the low values of $(c_1/c_p)^{1/3}$.

The influence of pressure can be analysed similarly. Because of the limited accuracy of the measurements, however, a whole range of parameters can give a good fit to the data of Fig. 2. The logarithm of the extremes of these values are plotted against P in Fig. 4b. Although it is not possible to calculate accurate values for the volume changes, it is clear that the pressure sensitivity is larger at 15 °C, and this is more pronounced for the parameter A than for the propagation equilibrium constant. This may again be explained by a conformational transition between the two temperature ranges.

These results give some indication of the type of bonds involved. The small pressure dependence at temperatures between 25 and 35 °C is probably the consequence of the dominance of hydrophobic interactions, which are usually associated with small volume changes; large volume changes are generally found in ionic interactions¹⁸. It is thus possible that lowering the temperature decreases the stability of hydrophobic interactions, and changes the conformation which leads to the exposure of charged groups. Independent evidence for this conformational change comes from the large difference in affinity of colchicine for the protomers, at 4 and 35 °C and from circular dichroism studies¹⁸. This conformational change seems

Fig. 3 Linearisation of the data of Fig. 1 using equation (2). ▼, -10 °C; □, -15 °C; ■, -20 °C; ○, -25 °C; ▲, -30 °C and ●, -35 °C.

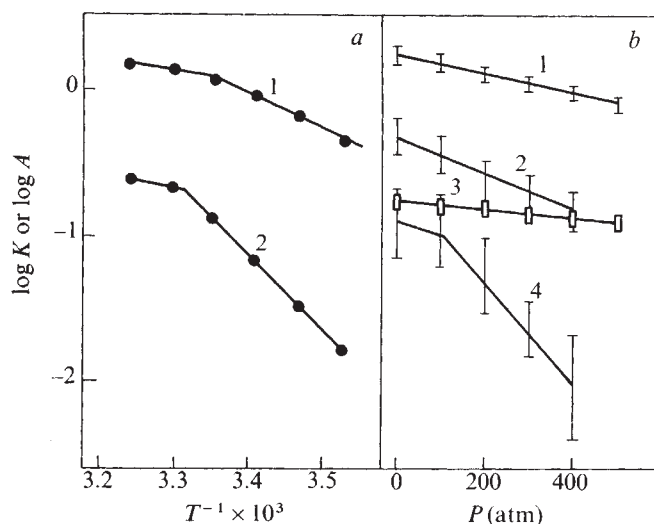
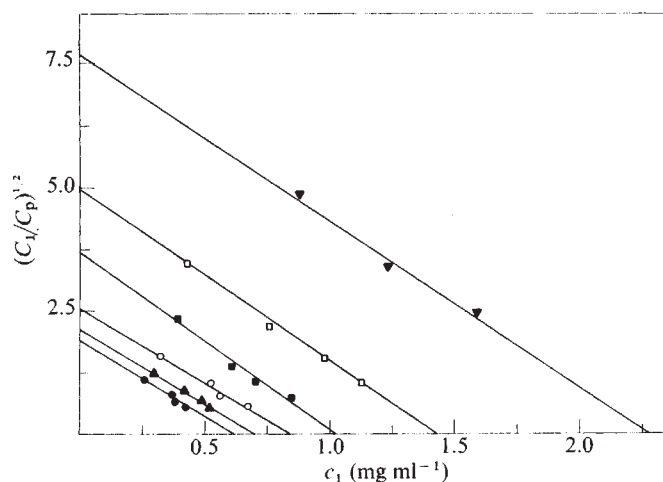


Fig. 4 a, Temperature dependence of the equilibrium constants. (1) For the propagation equilibrium constant K a value of $\Delta H = 10$ kcalorie mol^{-1} and $\Delta S = 34$ EU was found in the temperature range 25–10 °C. In the temperature range 35–30 °C, the values are halved. (2) For the nucleation parameter A the following values were obtained: $\Delta H = 25$ kcalorie mol^{-1} and $\Delta S = 80$ EU in the range 25–10 °C. At higher temperatures the values are about one-fifth of these. b, Pressure dependence of the equilibrium constants. Log K was plotted against P at 35 °C (1) and at 15 °C (2). The associated volume changes are 26 and 50 ml mol^{-1} respectively. Log A was plotted against P at 35 °C (3) and at 15 °C (4). The estimated volume changes are 12 and 120 ml mol^{-1} respectively.

to have its largest influence on the process of nucleation. It remains to be investigated whether other factors also exert their main influence on the nucleation equilibrium. This seems attractive, as a number of *in vivo* observations may be explained by the control of the nucleation process and the stability of the tube ends.

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Androgen-sensitive midbrain sites and visual attention in chicks

NEUROANATOMICAL networks within the preoptic-hypothalamic continuum of the avian brain contain neurones that are androgen-sensitive¹ and involved in the control of precocial copulation². In autoradiographic studies of the male chick brain, one of us (C.C.M.) has found midbrain structures, nucleus intercollicularis (ICo) and nucleus isthmo-opticus (IO) that concentrate radioactive testosterone or its metabolites. Neither of these structures has been implicated in the expression of sexual behaviour. Rather, there is evidence that both are involved in visual responsiveness that is not specifically associated with sexual behaviour. We wish to present the autoradiographic findings and relate them to the existing evidence of the functions of these two nuclei and testosterone in visual attention.

The technique has been described in detail elsewhere³. Male chicks were assigned to either an isolation ($n=3$) or isolation and testosterone primer ($n=3$) group. In the latter chicks 20 mg of testosterone propionate was subcutaneously injected on day 6 after hatching. On either day 15, 16 or 17 after hatching, male chicks received 200 μ Ci (1.3 μ g) of 1,2-³H-testosterone subcutaneously. Animals were decapitated 2 h after injection, their brains were removed, frozen in powdered CO₂, sectioned in a microtome-cryostat, and the sections were mounted on slides precoated with photographic emulsion. After exposure for 121-161 d, the autoradiograms were developed and stained with thionin. Quantitative analyses of reduced silver grains beneath neurones were conducted by reviewing 50 cell bodies for each neuroanatomical area surveyed in every animal. Results, which will be published in full elsewhere, showed high mean concentrations for silver grains in neurones of the ICo, both in isolated ($\bar{x}=13.77\pm1.26$ s.e.m.) and isolated and testosterone primed ($\bar{x}=10.09\pm1.07$ s.e.m.) subjects, which appear to be even greater than in the nucleus praeopticus paraventricularis magnocellularis ($\bar{x}=9.36\pm0.82$ s.e.m. isolates; $\bar{x}=6.68\pm0.73$ s.e.m., isolates and testosterone primer). This medial preoptic nucleus is known to contain androgen-sensitive neurones and to be involved in the development of precocial copulation in male chicks^{1,2}. In some animals autoradiograms extend into the posterior midbrain where the concentration of silver grains indicated a relatively high uptake of radioactive testosterone in the IO. Qualitative surveys for other midbrain areas, such as the tectum, nucleus mesencephalicus lateralis pars dorsalis, and central mesencephalic grey, did not show similar patterns of testosterone uptake. Control subjects receiving cold testosterone averaged no more than 1.80 grains per neurone in any brain region, and slides fogged with light before exposure showed no fading of the latent image. Although autoradiographic data from the songbird *Fringilla coelebs*⁴ also indicate that ICo is androgen sensitive, no other report has established that the IO contains androgen-sensitive cells.

Andrew reviewed studies of the ICo⁵. He points out that electrical stimulation of this nucleus induces calling and

alerting in chicks while lesions produce muteness and impaired visual targetting behaviour, including loss of scanning behaviour in an open field, and a reduction of inspection and absence of exploratory pecking of moving targets and 'novel objects'. Andrew and de Lanerolle⁶ have summarised these effects by saying that the lesioned chick "... ceases to treat visual (and probably other) stimuli as if they were conspicuous or highly valent". In previous studies Andrew⁷ found that testosterone injections in chicks produced, in addition to precocial copulation, an increase in persistence in attending to objects. His subsequent work⁸ has established that testosterone increases the probability of sustained visual attention to a particular stimulus or type of stimulus in competition with other distracting stimulation. In fact the effect seems to be the reverse of that produced by lesions of ICo so that the injected chick calls and orients to stimuli as if they were unusually conspicuous or valent. The facilitation of calling associated with the uptake of hormone by ICo may be part of the general attentional syndrome rather than the primary effect proposed by Zigmund *et al.*⁴, for Andrew¹⁰ has made the point that all chick calls may belong to a general set of attention behaviours expressing an attentional state. We suggest, therefore, that the ICo is part of a neural system for facilitating orientation behaviour. Such a system has been suggested by Salzen and Parker¹¹ to arise in the archistriatum which is responsible for facilitating the subtelencephalic systems that execute head and body orientations to objects in response to decisional commands from other striatal regions. Certainly ICo receives fibres from the archistriatum through the tractus occipitomesencephalicus. Andrew¹² has also outlined an archistriatal source of descending influence on the ICo through a diencephalic periventricular and central mesencephalic grey system and has proposed that the intercollicular area modulates the efferent flow from tectal systems for positive targetting, and/or enhances the tectal processes themselves. Thus, much of the chick lesion data can be understood in terms of tectal and brainstem systems for head and body orientation for visual fixation which are facilitated through an archistriatal-intercollicular pathway. The non-sexual behavioural effects of testosterone can then be seen as the results of selective facilitation of these tectal orientation systems, and the uptake of this hormone by the ICo could account for these effects.

The occurrence of a similar uptake of testosterone by the IO is interesting because this nucleus projects solely to the retina, and according to the electrophysiological studies of Miles¹³, activity in this nucleus effectively enhances retinal responses to both large and small stimuli by removing inhibitory surrounds and facilitating the excitatory centres of retinal ganglion cell response fields. Miles has suggested that the centrifugal system provides a rapid local transient dark-adaptation system for improved detection in shadowed areas during visual search. This is consistent with the findings of Rogers and Miles¹⁴, that lesions of the IO impaired the discrimination of food from sand grains in the darker areas of a variegated brightness field. These birds also pecked at food and non-food objects in an apparently indiscriminate and exploratory fashion with closed bill. They were also poorer at responding to a novel object introduced into the posterior visual field while feeding. These observations suggest an impairment of object recognition or target distinctiveness, permitting less selectivity within the attentional system and resulting in more general behavioural effects than would the loss of a local dark-adaptation system. We suggest, therefore, that androgen uptake in the IO increases target distinctiveness and so contributes to the observed enhanced visual orienting and attentional behaviour of the hormone-injected chick. The interest in both these midbrain nuclei being androgen sensitive is that they are parts of neural systems that could operate in orientation behaviour to specific focal visual stimulation

and so provide a neural basis for the perceptual and "non-sexual" behavioural effects of testosterone.

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Interspecific variation in products of animal mitochondrial protein synthesis

THE DNA of mammalian mitochondria (mtDNA) exists as a single class of circular molecules about 5 μ m long^{1,2}, and codes for two ribosomal RNA species and several transfer (4S) RNA components^{3,4}. The function of the remaining 80% of the genome is unknown, although it has been suggested that the residual coding capacity provides messenger RNA (mRNA) which is translated exclusively by mitochondrial ribosomes producing about ten protein components which are associated with the inner mitochondrial membrane. Not all protein synthesis in mitochondria, however, may be directed by mtDNA—some could represent translation products of nuclear-coded messages imported into the organelles^{5,6}. We report here on differences between electrophoretic profiles of proteins synthesised in mitochondria of a wider variety of animal cell lines. Evidence is also presented suggesting that mtDNA has a role in determining the pattern of proteins observed.

It is widely assumed that the proteins synthesised in cells treated with inhibitors of cytoplasmic protein synthesis are of mitochondrial origin. We have shown previously that about ten major proteins are selectively labelled by culturing cells in the presence of ³⁵S-methionine and the inhibitor emetine^{7,8}. The labelled proteins copurified with mitochondria on cell fractionation and their synthesis was blocked by chloramphenicol, a known inhibitor of mitochondrial protein synthesis. Our preliminary investigations demonstrated that the patterns for human and mouse mitochondrially-synthesised proteins, as resolved by electrophoresis in sodium dodecyl sulphate (SDS)-polyacrylamide gels, were different⁷.

A typical profile of the proteins detected by the technique of selective labelling followed by electrophoresis and autoradiography is illustrated in Fig. 1. This pattern obtained with human cells shows about eight predominant bands which were labelled in the presence of the cytoplasmic protein synthesis inhibitor emetine. The approxi-

mate molecular weights were determined by calibrating the gel with marker proteins of known size.

Using cells from various species, we have observed some differences in the profiles for animals more closely related than humans and mice. Thus, among the rodents (Fig. 2), profiles from rat and mouse cell lines were similar but showed small, reproducible differences from those of both hamster and rabbit. With other animal cells, there were variations in the electrophoretic mobilities of most labelled bands (Fig. 3). The total number of bands (7-11) and their molecular weight range (10,000-50,000), however, were fairly constant. Some of the components detected were common to profiles of most species examined—for example, the band of apparent molecular weight 45,000—whereas others varied considerably so that homologies were difficult to establish. The patterns for man, chimpanzee and the African green monkey showed an overall similarity, but differed somewhat from those of marmoset (a New World monkey). In all species studied, no more than about 3 μ m DNA would be required to code for these mitochondrially synthesised proteins (based on apparent molecular weight determinations). This is within the coding capacity of animal mtDNA. Bands of molecular weight above 50,000 were generally not sensitive to chloramphenicol. Furthermore, experiments with human cells demonstrated that

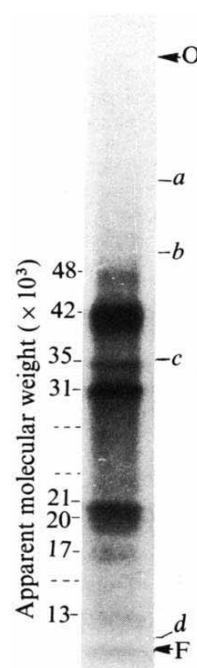


Fig. 1 Electrophoretic profile of proteins synthesised *in vivo* by mitochondria of human cells. HeLa cells, labelled with ³⁵S-methionine for 4 h in presence of 50 μ g ml⁻¹ emetine, were solubilised in buffer containing SDS and 2-mercaptoethanol. Samples were electrophoresed on a 12% (w/v) polyacrylamide slab gel and labelled polypeptides detected subsequently by autoradiography. Details of procedures for incorporation, electrophoresis and autoradiography have been described previously⁷. Marker proteins (a, bovine serum albumin; b, bovine glutamate dehydrogenase; c, rabbit lactate dehydrogenase; d, equine cytochrome c) were run on the same slab gel to provide a calibration for molecular weight determination. Labelled material of molecular weight about 42,000 consists of two closely spaced bands. Additional components which are sometimes detected in the system are marked by dashed lines. Addition of phenylmethylsulphonylfluoride (1 mM) before solubilisation was found not to affect band pattern observed. Errors in molecular weight assignment could result from differences in hydrophobicity between marker and mitochondrial proteins. O, Origin; F, front.

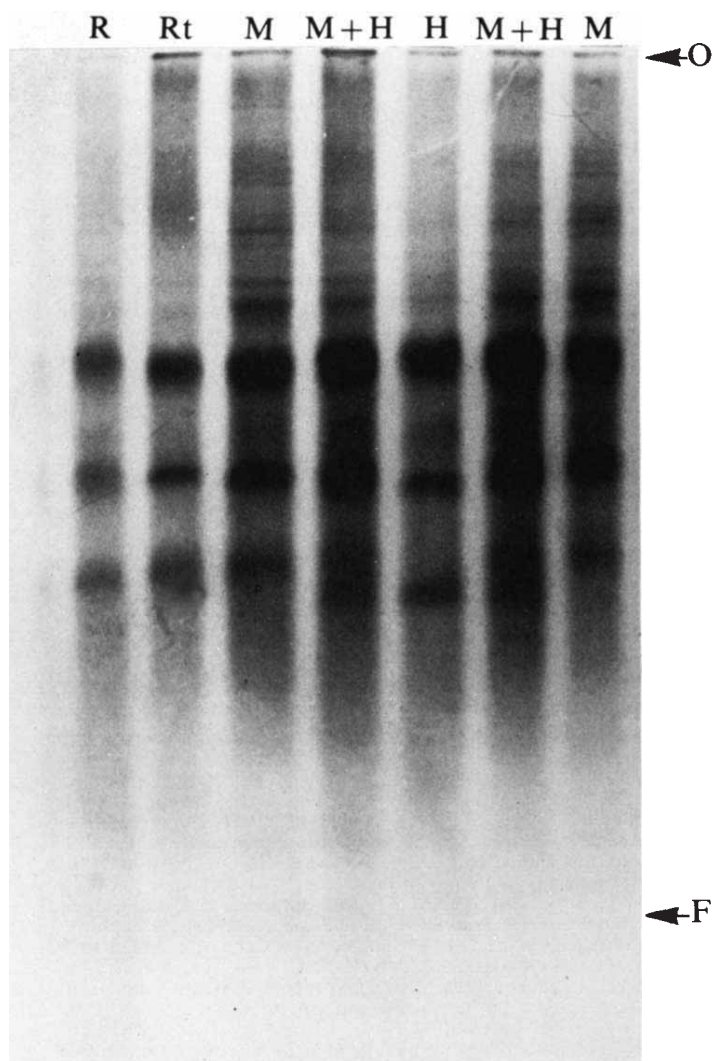


Fig. 2 Electrophoretic profile of mitochondrially-made proteins from various rodent cell lines. R, Rabbit; Rt, rat; M, mouse; H, Chinese hamster. Cells were labelled with ^{35}S -methionine for 4 h in presence of $50 \mu\text{g ml}^{-1}$ emetine, before solubilisation, electrophoresis and autoradiography. O, Origin; F, front.

they could be eliminated by purification of mitochondria before electrophoresis. These bands of higher molecular weight may represent the residual products of cytoplasmic protein synthesis.

We have also examined the spectrum of proteins synthesised by mitochondria of human-mouse somatic cell hybrids. Profiles obtained with 17 independently-derived hybrid cell lines were similar to those of mouse parental cell lines. No human components, which could be distinguished from the mitochondrially-synthesised proteins of the mouse, were detected. Similar observations on four such hybrid cell lines have been reported previously⁷. Karyotypic analysis indicated that every human chromosome (with the possible exception of the Y) was represented in at least one of the hybrids examined (ref. 9 and E. Solomon *et al.*, unpublished). Since human mtDNA seems to be eliminated from man-mouse hybrids in which the mouse parent is an established cell line^{10,11}, the detection of only mouse mitochondrially-synthesised proteins is consistent with the suggestion that the genes for at least some of the human proteins can be assigned to mtDNA. The absence of human mitochondrial proteins would thus reflect the loss of human mtDNA. Other interpretations based on the possible existence of dominant modification

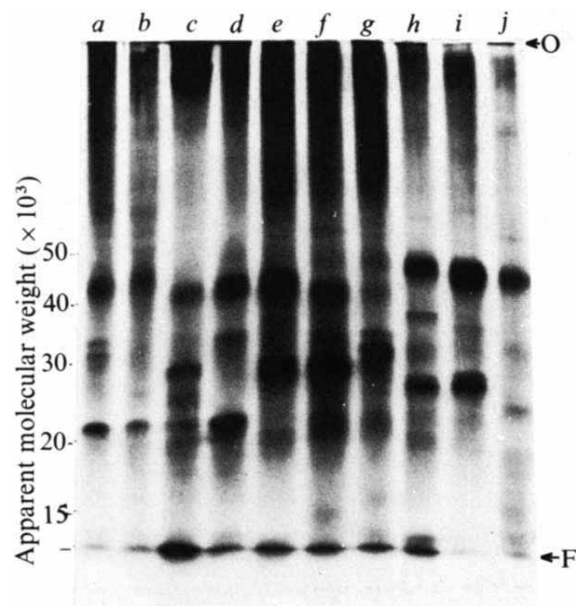


Fig. 3 An electrophoretic comparison of proteins synthesised by mitochondria of various animal cell lines. a, Man; b, chimpanzee; c, marmoset; d, African green monkey; e, cat; f, mouse; g, marsupial mouse (*Sminthopsis*); h, chicken; i, *Xenopus laevis*; j, *Drosophila melanogaster*. Cell lines were labelled for 4 h in presence of $100 \mu\text{g ml}^{-1}$ emetine. Incorporations were at 37°C except for *Xenopus* and *Drosophila* cells (25°C). Incorporation medium for *Drosophila* embryonic cells was D22 medium¹², in which yeastolate and lactalbumin hydrolysate were replaced by $67 \mu\text{M}$ L-amino acids minus methionine. Further supplements were 0.5% (v/v) foetal calf serum and 9.5% (v/v) dialysed serum⁷. Details of cell lines used may be obtained from the authors on request. O, Origin; F, front.

mechanisms controlled by genes on mouse chromosomes, or on the selective repression of human nuclear genes for these proteins, can be envisaged, but require further, as yet untested, assumptions.

The observed variation in the profiles of mitochondrially-synthesised proteins in the several species examined therefore may reflect divergence in the corresponding structural genes in the mitochondrial genome. In this context, animal mitochondria have been shown to synthesise about eight species of poly(A)-containing RNA, which seem to represent messages transcribed from mtDNA. Not only does this number of messages correspond roughly to the number of proteins in this study, but there is interspecies variation in the sizes of these RNA components^{12,13}. Furthermore, extensive divergence in the nucleotide sequences of mtDNA from various animal species has been reported¹⁴⁻¹⁶.

Our electrophoretic analysis indicates substantial variation in the size and/or composition of mitochondrially-synthesised proteins of different animal cell lines and a possible role of postsynthetic modification (for example, glycosylation, cleavage or aggregation) should be considered. Nevertheless, the reproducibility and stability of the profiles, together with information from the genetic analysis with human-mouse hybrid cells, suggests a lack of conservation of the proteins synthesised in animal mitochondria. Caution should therefore be exercised in extrapolations concerning their nature and function based on information from the lower eukaryotes—for example, yeast.

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Ribosome metabolism in temperature-sensitive mutant of BHK cells

CELLULAR content of ribosomes is coordinately regulated with cell growth¹, either at the level of ribosome processing or ribosomal degradation. The two ribosomal subunits in growing mammalian cells are synthesised in equimolar amounts as cleavage products of a common precursor²; and it also seems that all the components of ribosomes are degraded as a unit³. The regulatory mechanism of ribosome metabolism in mammalian cells, however, remains unclear.

We have studied ribosome metabolism in the temperature-sensitive mutant, *ts422E*, derived from BHK cells, that are defective in the processing of large ribosomal subunits (28S rRNA) at the non-permissive temperature (39 °C)^{4,5}. Synthesis of the small ribosomal subunits (18S rRNA), on the other hand, is uninhibited. The resulting transient imbalance in the ribosomal subunits at 39 °C seems to be corrected by a higher rate of degradation of the 18S rRNA in the cytoplasm. As the rate of accumulation of poly(A)-containing RNA in the polyribosomes, and the rate of protein synthesis in *ts422E* remain unchanged at 39 °C, it seems that the instability of a fraction of 18S rRNA in the mutant cells arises from their lack of participation in protein synthesis.

When shifted to the non-permissive temperature, the *ts422E* cells show a transient increase in cell number followed by an actual decline in viable cells compared with those cultures remaining at the permissive temperature (Fig. 2a, inset). Although the total ribosome content in *ts422E* cells show equimolar amounts of 28S and 18S rRNA after 25 h at 39 °C, the amounts of newly synthesised 28S rRNA at the non-permissive temperature is markedly reduced compared with cells at the permissive temperature (Fig. 1a and c). When the radioactivity in rRNA synthesised at 39 °C is chased with excess unlabelled uridine and cytidine for 42.5 h at 39 °C, however, the specific activities of 28S and 18S rRNA approach that of *ts422E* cells maintained at the permissive temperature (Fig. 1b and d). Therefore, the 18S rRNA accumulated in *ts422E* soon after shift up to 39 °C must be balanced by its faster rate of degradation. This possibility was tested directly by comparing the rate of decay of 18S and 28S rRNA in *ts422E* cells at the permissive and non-permissive temperatures.

The radioactivity in 18S rRNA from *ts422E* cells labelled

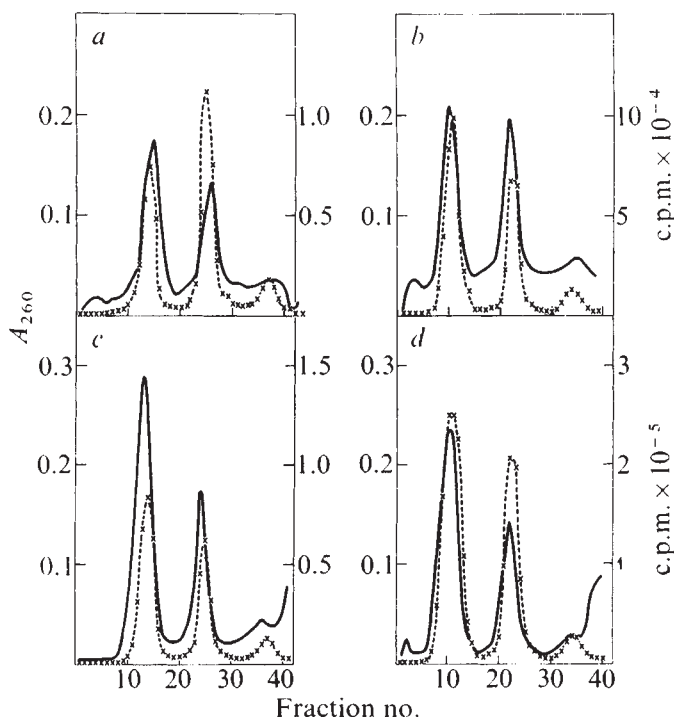
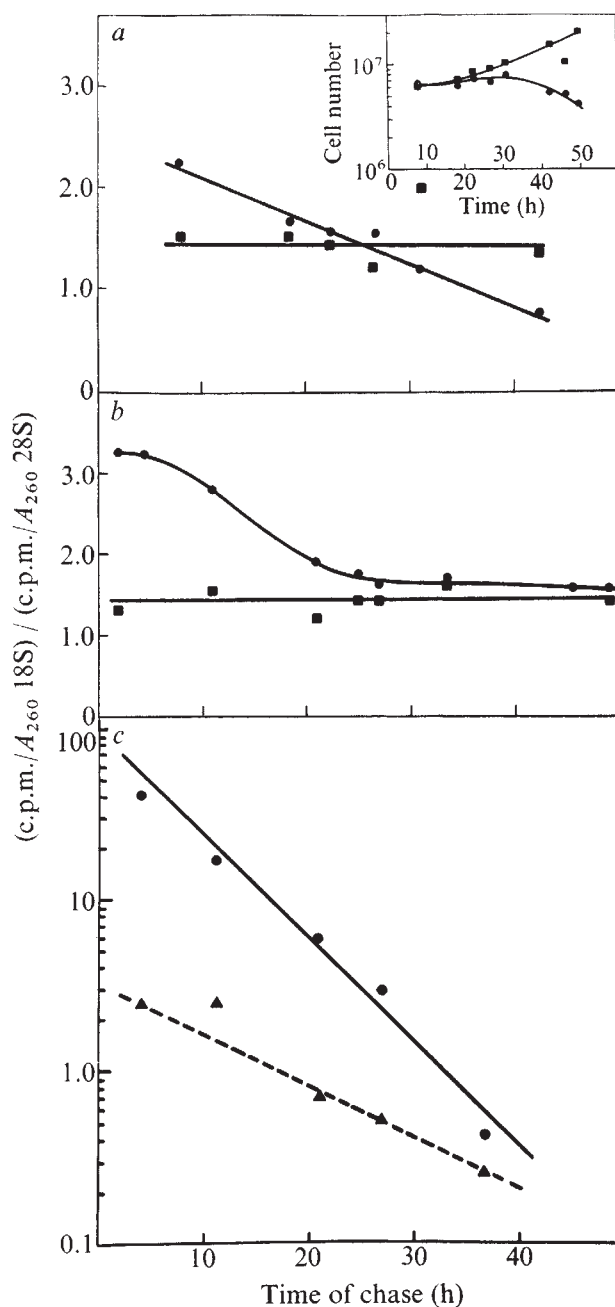


Fig. 1 RNA synthesis in *ts422E* at 39 °C. Cultures of *ts422E* cells were maintained at 33 °C in Dulbecco-Vogt modified Eagle's Medium (DME) supplemented with 10% calf serum. Eight 100-mm culture dishes were shifted to 39 °C and labelled between 15 and 17 h with 10 μ Ci ml⁻¹ ³H-uridine (specific activity 25 Ci mmol⁻¹). After ³H-uridine incubation, labelled RNA was chased by substituting the medium with fresh DME supplemented with 5 mM uridine plus 2.5 mM cytidine at 39 °C for 8 h (a) and 42.5 h (b). Control cultures maintained at the permissive temperature were pulse-labelled and chased as above (c and d). The chase was terminated by washing cells with chilled (2–4 °C) balanced salt solution (Earle's). Cells were detached by trypsin treatment, washed twice with cold Earle's solution and disrupted by Dounce homogenisation in RSB buffer (0.01 M Tris-HCl, pH 7.6, 0.01 M NaCl, 1.5 mM MgCl₂). Nuclei and large cell particulates were eliminated by successive centrifugations at 1,000g for 3 min and 12,000g for 10 min, and the ribosomes were precipitated from the postmitochondrial supernatant with the addition of 70 mM MgCl₂ for 45 min at 4 °C (ref. 10). Ribosomes were pelleted by centrifugation at 12,000g for 10 min and the pellets resuspended in 5 ml NETS buffer (0.01 M Tris-HCl, pH 9.0, 0.01 M EDTA, 0.1 M NaCl, 0.2% SDS) and the RNA extracted with equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) until no visible interphase was present. RNA was precipitated from the aqueous phase with two volumes of ethanol, collected by centrifugation and resolved by centrifugation in 15–30% sucrose gradients in NETS buffer. Samples were centrifuged in a Spinco SW27 rotor at 23 °C and 23,000 r.p.m. for 16 h. Gradients were eluted through a recording spectrophotometer, and samples were prepared for the determination of acid-insoluble radioactivity and counted in a liquid scintillation counter as described¹⁰. a, 8-h chase at 39 °C; b, 42.5-h chase at 39 °C; c, 8-h chase at 33 °C; d, 42.5-h chase at 33 °C. —, A_{260} ; x, c.p.m.

with ³H-uridine between 15 and 17 h at 39 °C, decays at a faster rate than the radioactivity in 28S rRNA when chased in a growth medium containing excess unlabelled uridine and cytidine at 39 °C. During a similar chase period of 40–50 h there is little difference in the relative rates of decay of 18S and 28S rRNA of the mutant cells that remained at the permissive temperature (Fig. 2a). The enhanced decay of 18S rRNA in the temperature-sensitive mutants seems to be a transient adjustment to the non-permissive condition. As shown in Fig. 2b, the faster decay rate of ³H-methylmethionine pulse-labelled 18S rRNA at 39 °C, on being chased with excess unlabelled methionine at 33 °C, approaches the rate of rRNA degradation in the cells that were labelled and chased at the permissive temperature. This apparent recovery from the faster rate of

Fig. 2 Rates of decay of 18S and 28S rRNA. *a*, *ts422E* cells maintained at 33 °C were shifted to 39 °C and pulse labelled with ^3H -uridine between 15 and 17 h as described in Fig. 1. Control cultures maintained at 33 °C were similarly pulse labelled for 2 h. After pulse label, the growth medium was replaced with DME containing excess unlabelled uridine and cytidine, and cells collected at indicated times after the chase. rRNA from cells after various chase periods was fractionated, and the 18S and 28S rRNA resolved on NETS-sucrose gradients as in Fig. 1. Specific activities were determined by integrating the areas under the A_{260} profiles of 18S and 28S rRNA, and adding the radioactivities of the two rRNA species. The change in the relative specific activities of rRNAs for cultures kept at 33 °C and those at 39 °C are shown: ■, 33 °C; ●, 39 °C. Inset shows changes in cell number of the cultures maintained at 33 °C and 39 °C. *b*, Cultures of *ts422E* cells were shifted to 39 °C in normal growth medium and pulse labelled with 25 $\mu\text{Ci ml}^{-1}$ ^3H -methylmethionine (specific activity 300 mCi mmol^{-1}) for 30 min (between 16.5 and 17 h at 39 °C) in DME lacking methionine but containing 10% calf serum. The medium was then replaced with a chase medium (DME containing 8 mM methionine plus 10% calf serum), and labelled cells restored to the permissive temperature and allowed to grow at 33 °C. Control samples *ts422E* cells maintained at 33 °C were similarly pulse labelled with ^3H -methylmethionine and also chased at 33 °C. At indicated times after the beginning of the chase period, samples from cells pulse labelled at 39 and 33 °C were removed. Cells collected and rRNA purified and resolved on sucrose gradients as in Fig. 1. Relative rates of decay in the specific activities of rRNAs were estimated by integrating the areas under the A_{260} profile and the radioactivity under the 28S and 18S rRNA, as in *a*. *c*, Ten 100-mm culture dishes of *ts422E* cells were labelled with ^{14}C -methylmethionine (5 $\mu\text{Ci ml}^{-1}$, specific activity 50 mCi mmol^{-1}) for 24 h at 33 °C in DME plus 10% calf serum. After steady-state labelling with ^{14}C -methionine, the medium was replaced with fresh DME and the cells grown for the first 16 h at 33 °C and then shifted to the non-permissive temperature (39 °C) for another 16 h. Cells were then pulse labelled with 20 $\mu\text{Ci ml}^{-1}$ ^3H -methylmethionine (specific activity 230 mCi mmol^{-1}) for 1.5 h at 39 °C and chased with fresh DME supplemented with 10% calf serum and 8 mM L-methionine. Samples were taken at indicated times after the beginning of chase at 39 °C; cells were collected and rRNAs prepared and resolved on sucrose gradients as above. The radioactivity in each rRNA species was normalised to the A_{260} of total cell DNA prepared according to Scott *et al.*¹¹. The illustration compares the relative rate of decay of specific activities in rRNAs labelled before (^{14}C) and after (^3H) the shift up to non-permissive temperature.



decay of rRNA at 39 °C is complete within about 24 h of restoration of the permissive growth conditions (Fig. 2b). Since the experiments so far have shown an accelerated rate of decay of 18S rRNA after a shift up to 39 °C, it is of interest to examine whether the 18S rRNA synthesised at the non-permissive temperature is preferentially degraded. Although both the existing 18S rRNA (^{14}C -labelled) synthesised at the permissive temperature and that synthesised after the shift up to 39 °C (^3H -labelled) are degraded at a faster rate than the corresponding 28S rRNA, the rate of decay of 18S rRNA made after the shift to 39 °C was

Table 1 Appearance of 18S rRNA in cytoplasm of BHK and 422E cells

Time	New		Polyribosomal Old		Ratio		New		Native subunits Old		Ratio	
	$^3\text{H}(\text{c.p.m.}/10^7 \text{ cells})$	$^{14}\text{C}(\text{c.p.m.}/10^7 \text{ cells})$	BHK	422E	BHK	422E	BHK	422E	BHK	422E	BHK	422E
30 min	—	72	—	213	—	0.33	437	508	770	929	0.56	0.83
60 min	395	192	243	220	1.62	0.87	2,052	1,871	886	506	2.31	3.70
90 min	1,085	3,060	381	1,257	2.85	2.43	8,452	2,866	1,256	493	6.73	5.81
24 h	11,189	4,654	2,105	1,370	5.32	3.39	5,080	5,622	763	1,023	6.65	5.50

Proportions of old and new 18S rRNA within polysomal and native subunit fraction of BHK and 422E cells determined by incubating cultures of both types of cells at permissive temperature with 2.5 $\mu\text{Ci ml}^{-1}$ ^{14}C -methylmethionine (specific activity 58 mCi mmol^{-1}) in complete medium for 24 h. Growth medium then replaced twice within 8 h with fresh DME at 33 °C and cultures transferred to 39 °C. After 17 h at non-permissive temperature, cells pulse labelled with 25 $\mu\text{Ci ml}^{-1}$ ^3H -methylmethionine in DME lacking methionine and samples removed at 30, 60 and 90 min thereafter. Remaining fraction of each of labelled BHK and *ts422E* cultures washed and grown for 24 h in chase medium containing excess (40 times normal concentration in DME) unlabelled methionine. Cells collected, homogenised and postmitochondrial supernatant resolved through 8 ml 5–20% (w/w) sucrose gradient in RSB, layered over a 2-ml cushion 60% sucrose in RSB, and centrifuged in a Spinco SW41 rotor at 36,000 r.p.m. for 3.5 h at 4 °C. Polysomal and native 40S subunit regions collected and RNA phenol extracted as before, purified by oligo-d(T)-cellulose chromatography, and poly(A)-lacking RNA further resolved on 15–30% sucrose-NETS gradients as in Fig. 1. Radioactivity in old (^{14}C) and new (^3H) 18S rRNA from each sample corrected for background and spill, normalised to number of cells in each fraction.

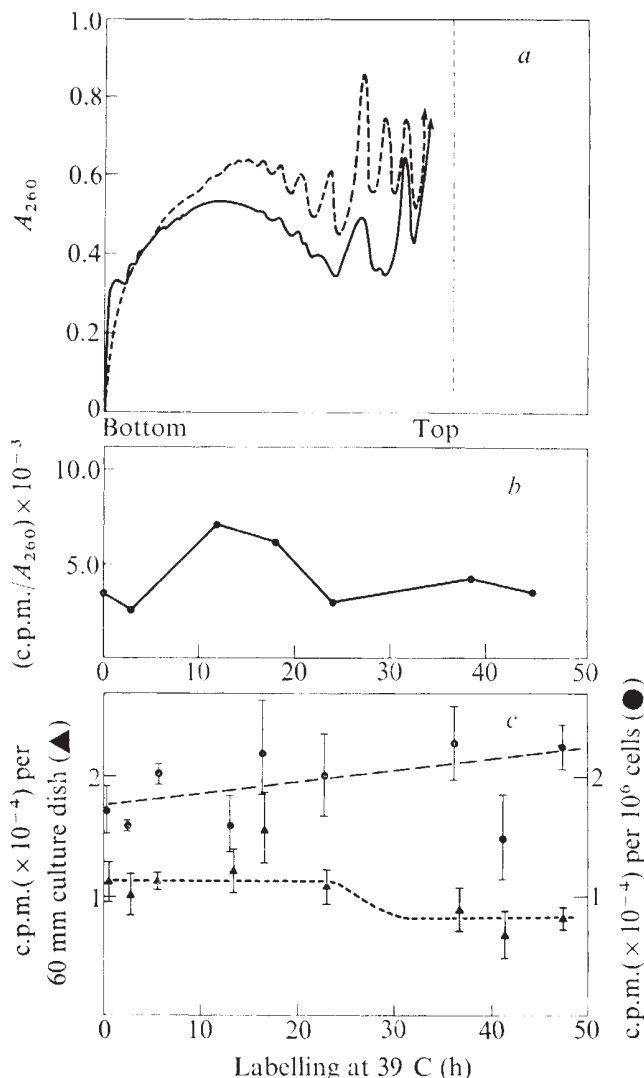


Fig. 3 Polyribosomal RNA and protein synthesis in *ts422E* at 39 °C. **a**, Cultures *ts422E* cells maintained at 33 °C shifted to 39 °C for 3 h (broken line) and 24 h (solid line). Cells were collected, washed and disrupted by Dounce homogenisation in RSB buffer as described¹⁰. Postmitochondrial supernatant was prepared by centrifugation at 12,000*g* for 10 min, and polyribosomes from the supernatant resolved by centrifugation in 15–30% (w/w) sucrose gradient in RSB. Centrifugation was done in a Spinco SW27 rotor at 26,000 r.p.m. for 2.5 h and 4 °C, and the sample eluted through a continuously recording spectrophotometer. A_{260} ; —, 3 h at 39 °C; - - - - - , 24 h at 39 °C. **b**, Polysomal poly(A)-containing RNA was estimated from cultures labelled for 2 h with ³H-adenosine (50 $\mu\text{Ci ml}^{-1}$, specific activity 40.8 Ci mmol⁻¹) before isolating polyribosomes as in **a**. Pooled polyribosomes were extracted twice with phenol-chloroform-isoamyl alcohol (50:50:1) followed by extraction with chloroform-isoamyl alcohol (96:4) and precipitated with two volumes of 95% ethanol. A_{260} was determined on sample aliquots. Incorporation of adenosine into polysomal poly(A) was measured by incubating samples with T₁ RNase (20 U ml⁻¹) and RNase A (20 $\mu\text{g ml}^{-1}) at 37 °C for 20 min in 0.5 M NaCl and 0.01 M Tris-HCl buffer. The mixture was run through an oligo(dT)-cellulose column and washed extensively with the same buffer. The amount bound was determined by eluting poly(A) with 0.1 M Tris, pH 7.4, buffer and determining the c.p.m. in the eluate. ●, c.p.m. in polysomal poly(A)/ A_{260} of polysomal RNA. **c**, Rate of protein synthesis was determined in 60-mm cultures of *ts422E* cells shifted to 39 °C and pulse labelled with 2 $\mu\text{Ci ml}^{-1}$ ³H-L-amino acid mixture (NET-250) for 1 h. Each point represents an average of three determinations (vertical bar represents the range of counts in each of the three 60-mm cultures). The first time point was taken soon after the shift from 33 to 39 °C and the subsequent ones at indicated time intervals after transfer to 39 °C. Incorporation was terminated by the addition of 5 ml cold (2–4 °C) balanced salt solution, and cells collected and washed as before. Cells were then lysed and prepared for the determination of acid-insoluble radioactivity as described¹⁰.$

faster than that of the pre-existing 18S rRNA (Fig. 2c).

As shown in Fig. 3a, there is an increase in the amount of free 40S subunits, which presumably represents the excess 18S rRNA not yet utilised in protein synthesis. Since there is no shift towards slower-sedimenting polyribosomes at the non-permissive temperature (Fig. 3a), it is likely that the increased quantity of free 40S subunits in *ts422E* after 24 h at 39 °C represents an inhibition of translational initiation.

The rate of accumulation of poly(A)-containing (poly(A)⁺) pulse-labelled polyribosomal RNA is *ts422E* (Fig. 3b) is not significantly altered after 40 h at the non-permissive temperature. The initial increase in the accumulation of poly(A)⁺ polyribosomal RNA may represent an enhanced rate of incorporation in acid-precipitable RNA at 39 °C compared with 33 °C since there is no change in the acid-soluble pool at the two temperatures (A.K., E.B. and W.T.M., unpublished). Thus it seems that there is little change in the rate of messenger RNA (mRNA) processing in the temperature-sensitive mutants at the non-permissive temperature.

The rate of protein synthesis in *ts422E* cells is slightly increased when shifted from 33 to 39 °C (Fig. 3c), an effect similar to that of increased incorporation of acid-precipitable RNA at 39 °C. On a per-culture basis, however, there is a slight decline in the rate of protein synthesis after about 25 h at 39 °C, largely because of the loss in the number of viable mutant cells on prolonged incubation at 39 °C (Figs 3c and 2a, inset).

The fact that pre-existing small ribosomal subunits are degraded at a slower rate at 39 °C than those synthesised after shift up to the non-permissive temperature (Fig. 2c) may suggest that the pre-existing ribosomes, having been used in protein synthesis, are more stable than the nascent ribosomes.

An estimate of the amounts of existing 18S rRNA and the new 18S rRNA synthesised at 39 °C in the polyribosomal and native subunit fractions (Table 1) further suggests that more existing 18S rRNA enters the polyribosomes in *ts422E* cells at the non-permissive temperature than in the wild-type cells within 90 min of equilibration.

During the transition from resting to growing fibroblasts there is a marked increase in the stability of rRNA^{6,7}. One of the early events in the re-initiation of cell growth seems to be an increased use of ribosomes^{8,9}, indicated by the enhanced rate of protein synthesis. Since the two ribosomal subunits are functionally coupled in mRNA translation, it seems that the stability and thus the control of degradation of ribosomes in mammalian cells depends on their use in protein synthesis.

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Magnitude and significance of NAD turnover in human cell line D98/AH2

In the oxidation-reduction metabolism of a cell the pyridine nucleotide NAD is used catalytically; any NAD that is reduced is reoxidised. But NAD is consumed in certain metabolic reactions in which it serves as a substrate. In eukaryotic cells, the most intriguing of these reactions is the cleavage of NAD to form nicotinamide and a unique polymer, poly adenosine diphosphoribose (poly ADPR) (Fig. 1); the reaction is catalysed by the enzyme poly ADPR synthetase¹⁻⁴. Other well studied reactions involving the destruction of NAD include the cleavage of NAD to form AMP and NMN by bacterial DNA ligases^{5,6} and the breakdown of NAD (with the concomitant inactivation of protein synthesis) by diphtheria toxin^{7,8}.

What is the magnitude *in vivo* of the reactions which consume NAD? We present here data for D98/AH2, a human cell line derived from HeLa⁹ which answers this question and which makes possible calculation of the fraction of NAD biosynthesis that compensates for breakdown and the fraction that expands the NAD pool during growth.

D98/AH2 cells were grown in medium containing ¹⁴C-adenine and ³H-nicotinic acid, both precursors of NAD, for 6 h and then moved to unlabelled medium. At various times after transfer, intracellular nucleotides were extracted with acid and analysed by paper chromatography. The data obtained from one such experiment are shown in Table 1. These data permit a precise calculation of the breakdown rate of NAD *in vivo* (Table 2). Several experiments of this general type have been performed; all yield values for the *in vivo* half life of NAD of about 1.0 ± 0.3 h for D98/AH2 cells¹⁰. This value is equivalent to a breakdown rate of $96,000 \pm 30,000$ per s per cell. Because the generation time of D98/AH2 is 24 h and the NAD pool size is 5×10^8 molecules per cell (ref. 11), a net synthesis

Table 1 Relative amount of ¹⁴C-adenine in ATP and NAD pools as a function of time

Time (h)	¹⁴ C-adenine in:	
	NAD pool (D_t/D_0) (expressed as a ratio to the radioactivity at $t=0$)	ATP pool (A/A^0)
0	1.0	1.0
1	0.87	0.86
3	0.70	0.47
8	0.39	0.24

Four equivalent D98/AH2 cultures ($\sim 2 \times 10^6$ cells) were grown in 5 ml Falcon flasks for 8 h in F12 medium which contained $0.2 \mu\text{g ml}^{-1}$, nicotinic acid ($0.1 \mu\text{g ml}^{-1}$, 5×10^8 c.p.m. μmol^{-1}) and ¹⁴C-adenine ($0.2 \mu\text{g ml}^{-1}$, 6.6×10^7 c.p.m. μmol^{-1}); cells were then washed and allowed to grow in unlabelled F12 medium which contained nicotinic acid $0.1 \mu\text{g ml}^{-1}$ and adenosine ($20 \mu\text{g ml}^{-1}$) for the time indicated. Nucleotides were acid-extracted from the cells and analysed by chromatography on DEAE paper as described previously¹¹. The total ¹⁴C in the ATP peak and ³H and ¹⁴C in the NAD peak was calculated for each chromatogram. The ¹⁴C-adenine in the ATP and NAD pools could then be determined, using the ³H in NAD to normalise to time (t) zero. The amount of radioactivity in each pool for the culture collected at $t=0$ is: ¹⁴C-adenine in ATP, 159,000 c.p.m.; ¹⁴C-adenine in NAD, 21,000 c.p.m.; ³H-nicotinamide in NAD, 165,000 c.p.m.

rate of only 4,000 molecules per s per cell is needed to maintain the pool size of NAD during growth. Thus, the true rate of NAD biosynthesis is approximately 10^3 molecules per s per cell and about 95% of this replaces the NAD that is catabolised, and only 5% maintains the NAD pool size during growth.

We have already studied NAD turnover in enucleated D98/AH2 cells¹². Cells were grown in ³H-nicotinic acid, enucleated by cytochalasin treatment and centrifugation¹³, and then incubated in unlabelled medium. The rate of loss of ³H-NAD was measured by an autoradiographic method developed for pyridine nucleotides¹⁴. The observed half life of the labelled NAD in enucleated cells was 10 h (ref. 12). Because enucleated cells cannot synthesise NAD¹², the observed 10 h half life of the label is a direct measure of

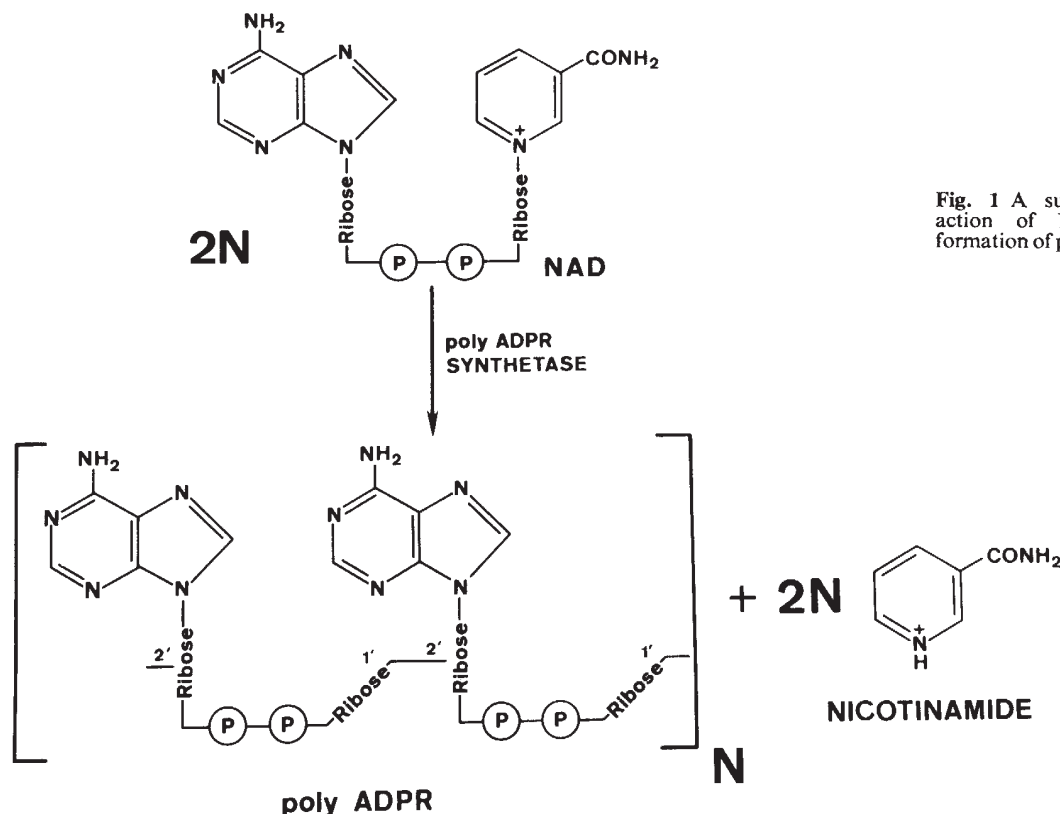


Fig. 1 A substrate reaction of NAD: the formation of poly ADPR.

Table 2 Constants calculated from the experimental data in Table 1

	Adenine in NAD (D)	Adenine in ATP (A)
Apparent decay constant, K	$3.3 \times 10^{-5} \text{ s}^{-1}$	$5.2 \times 10^{-5} \text{ s}^{-1}$
Apparent half life	5.8 h	3.7 h

True rate of breakdown of NAD, R_B : 124,000 molecules per s per cell
 True half life for adenine in NAD, τ : 0.78 h
 R_B was estimated by the equation:

$$\ln D_t/D_0 = -\frac{R_B t}{n_D} + \frac{(R_A + R_B)\{1 - \exp[-(K_A - K_D)t]\}}{(K_A - K_D)}$$

where D_t , D_0 are the radioactivity of adenine in the NAD pool at times t and 0 respectively, and K_A and K_D are the decay constants above, n_D is the pool size of NAD (no. of molecules per cell), R_A the number of NAD molecules synthesised to increase NAD pool size during growth. The quantity $(R_A + R_B)$ therefore represents the total rate of NAD synthesis. A derivation of this equation with a complete kinetic analysis of this and similar data is represented elsewhere¹⁰.

$$\tau = \frac{0.693 n_D}{R_B}$$

the rate of breakdown of NAD. As described above, NAD has a 1 h half life in nucleated cells. Together, these observations strongly indicate that NAD breakdown occurs primarily in the nucleus, although other more complex possibilities cannot be rigorously excluded.

The picture begins to emerge of the nucleus as an organelle in which NAD is actively synthesised and broken down. It has been known for many years that NAD pyrophosphorylase, one of the key NAD biosynthetic enzymes, is located within the nucleus¹⁵. Indeed, this enzyme is used as the characteristic marker enzyme of chromatin¹⁶. The data reported here, which indicate that most NAD biosynthesis compensates for NAD breakdown in the nucleus provide a rationale for the well established location of this NAD biosynthetic enzyme.

In isolated nuclei, poly ADPR synthetase is the major enzyme causing NAD breakdown^{3,4}. Although direct and conclusive evidence is not available, we suggest that this also holds true in the intact cell. Several features of the reaction catalysed by the synthetase suggest that the enzyme may be involved in nuclear function. The enzyme requires DNA and histones for activity¹⁷. The product of the reaction, poly ADPR, which incidentally, is both polynucleotide and polysaccharide, has been reported to be covalently bound to histone F1 (ref. 18). The synthetase has been found in all types of eukaryotic cells, from the slime mould *Physarum*¹⁹ to human tissue culture cells; this is consistent with a vital (and therefore evolutionarily conserved) role for the enzyme in the eukaryotic nucleus.

Our measurements indicate a surprisingly high rate of NAD breakdown; during one cell cycle, for example, there is twice as much adenine leaving NAD as is being incorporated into DNA. If poly ADPR synthesis were indeed responsible for a substantial fraction of NAD breakdown, then the amount of poly ADPR synthesised in one cell cycle would be of the same order of magnitude as the amount of DNA synthesised. A high steady-state level of poly ADPR, however, would not necessarily accumulate if breakdown were rapid. Indeed, two enzymes able to degrade poly ADPR have been isolated from mammalian nuclei^{20,21}.

The existence of a cellular NAD turnover cycle was first proposed by Gholson in 1966 (ref. 22). He argued that such a cycle "... had an important but as yet unknown function ... in cellular metabolism". This statement still applies; it seems likely that in eukaryotic cells, NAD has some other major function in addition to the classical cytoplasmic role in oxidation and reduction (which utilises

the nicotinamide moiety). Our results indicate that most NAD is synthesised to be consumed in a still poorly understood nuclear NAD metabolism, in which the non-nicotinamide (ADPR) part of the NAD molecule may have the major role.

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Translation of oncogenic virus RNA in *Xenopus laevis* oocytes

OOCYTE injection as developed by Gurdon *et al.*¹⁻³ is efficient and reliable for the assay of faithful translation of heterologous messenger RNA²⁻⁶. But, working in collaboration with Gurdon⁷, we found that oncogenic viral RNA was not translated to a detectable extent in oocytes. Only in a cell-free system derived from *Escherichia coli* could we translate viral RNA into distinct polypeptides in the molecular weight range of the viral group-specific proteins⁸. Recently we achieved translation of Rauscher leukaemia virus (RLV) RNA and avian myeloblastosis virus (AMV) RNA in various cell-free systems, using immunoprecipitation for the analysis^{9,10}. This verified that our failure to find virus-specific polypeptides in the oocyte system programmed with oncogenic viral RNA was due to the inadequacy of the methods available to detect translation when it occurs at low efficiency. We now report the synthesis of AMV-specific precursors and structural proteins in the oocyte system, detected by specific immunoprecipitation and scintillation autoradiography.

AMV contains single stranded RNA encapsulated by several proteins and a lipid envelope. The four major proteins, the so-called group-specific (gs) antigens immunologically indistinguishable among all avian oncornaviruses¹¹, are within the viral envelope¹².

There are additional glycosylated proteins, carrying the type-specific (ts) antigens, on the surface of the virion¹³. It is not known how many proteins are encoded by the viral RNA and how many cellular proteins become encapsulated in the virions during budding. To determine virus-specific proteins it is important to characterise the coding capacity

of the viral RNA in conditions closely related to the *in vivo* situation.

The results of a typical translation experiment of AMV RNA are shown in Fig. 1. Because of the relatively high rate of endogenous protein synthesis of this system, immunoprecipitation with specific antisera is a prerequisite for

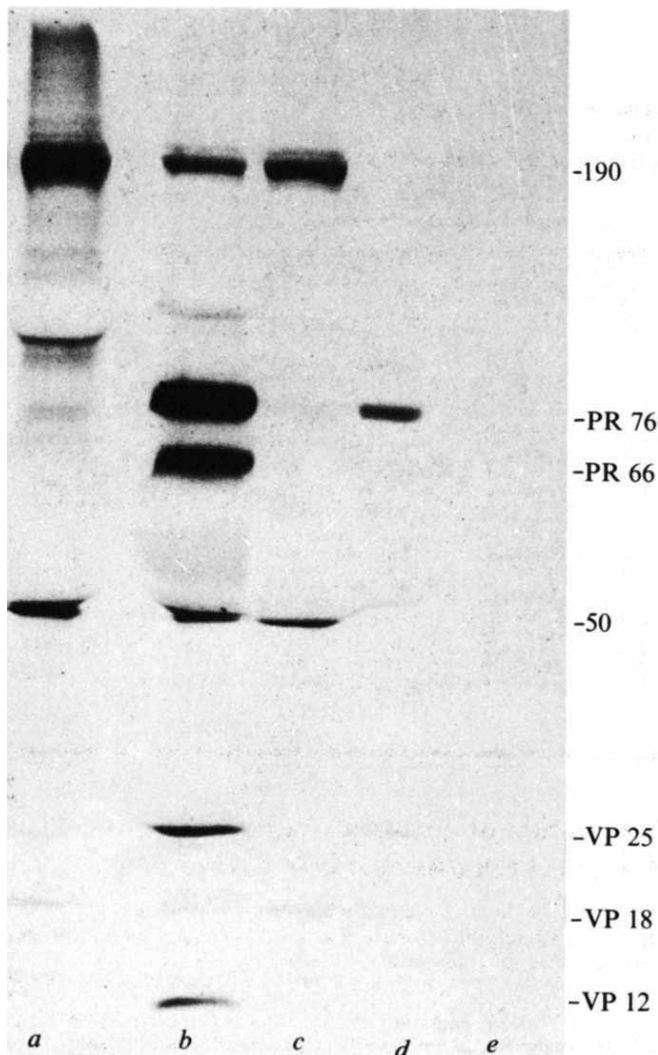


Fig. 1 Autoradiography of an SDS gel electrophoresis pattern of immunoprecipitates. *Xenopus laevis* oocytes were injected by the method of message-injection described by Gurdon *et al.*¹. AMV was obtained from viraemic plasma¹⁵ and treated with SDS and Pronase. The 60S component of AMV RNA was isolated by centrifugation in isokinetic glycerol gradients⁸. Isolated 60S AMV RNA was denatured by heating at 100 °C for 3 min. The AMV-specific polypeptides synthesised in the oocytes in the presence of 2 μ Ci ³⁵S-methionine (specific activity 478 mCi mmol⁻¹) per oocyte were detected by indirect immunoprecipitation¹⁶ with an antiserum directed against the gs antigens of AMV. Primary cultures of 1×10^6 chick embryo fibroblasts infected with AMV were pulse labelled for 30 min with 50 μ Ci ³⁵S-methionine (specific activity 210 Ci mmol⁻¹) in Earle's saline. After lysis in buffer indirect immunoprecipitation was carried out to detect virus-specific polypeptides. The immunoprecipitates were analysed on sodium dodecyl sulphate (SDS)-polyacrylamide slab gel gradients (7–18%) according to Berns *et al.*¹⁷. After staining and destaining, the slab gel was treated with DMSO-PPO¹⁸, dried under vacuum and exposed in contact with a Kodak X-ray film for 3 d. *a*, Each of 20 oocytes was injected with 25 nl of sterilised distilled water; *b*, each of 10 oocytes was injected with 25 nl of native 60S AMV RNA (1 mg ml⁻¹) in distilled water; *c*, each of 10 oocytes was injected with 25 nl of heat-denatured 35S AMV RNA (1 mg ml⁻¹); *d*, immunoprecipitate from pulse-labelled chick embryo fibroblasts infected with AMV; *e*, immunoprecipitate from pulse-labelled uninfected chick embryo fibroblasts. PR, precursor polypeptide; VP, virion polypeptide. Numerals denote molecular weight in kdaltons.

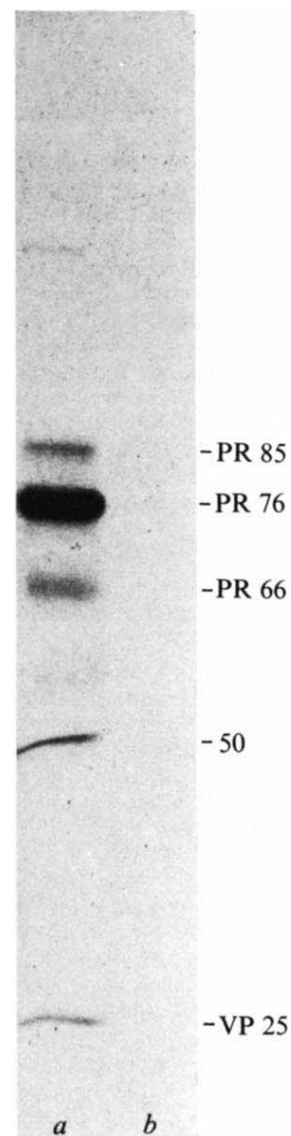


Fig. 2 Autoradiograph of an SDS gel electrophoresis pattern of immunoprecipitates. Primary cultures of 1×10^7 chick embryo fibroblasts either uninfected or infected with AMV were pulse labelled for 30 min and immunoprecipitated as described in Fig. 1. The immunoprecipitates were analysed on slab gel gradients and exposed in contact with a Kodak X-ray film for 3 d. *a*, 1×10^7 infected cells precipitated with anti-AMV serum; *b*, 1×10^7 uninfected cells precipitated with anti-AMV serum.

detection of the viral proteins. Injection of native 60S AMV RNA results in synthesis of virus-specific polypeptides with molecular weights of 76,000, 66,000, 32,000, 25,000, 18,000 and 12,000. In contrast, denatured 35S AMV RNA leads to the synthesis of a small quantity of the precursor polypeptide with molecular weight 76,000. In chick embryo fibroblasts (CEF) infected with AMV the same precursors and structural polypeptides are found as have been synthesised after injection of oocytes with native 60S AMV RNA (compare Fig. 1b and d).

In addition to the five precursor polypeptides already described¹⁴ we found in AMV-infected CEF a sixth unstable precursor of molecular weight 85,000 not present in uninfected CEF (Fig. 2). This pr85 was, however, not detectable after injection of AMV RNA into oocytes. Incubation of native 60S AMV RNA in a reticulocyte cell-free system (Fig. 3) resulted in the synthesis of the same precursors and structural polypeptides found in the oocyte system, but in

different proportions (compare Figs 1 and 3). In this cell-free system pr76 is the predominant product and almost no cleavage products are present. This probably means that the processing of AMV precursors in the oocyte and in infected CEF is almost identical, whereas in the reticulocyte cell-free system almost no cleavage occurs. In other reticulocyte preparations processing of the 76,000-dalton precursor resembled the situation *in vivo* (Fig. 4). Whereas the synthesis of the 76,000-dalton precursor polypeptide in a reticulocyte cell-free system is detectable even without immunoprecipitation (Fig. 3), as a rule immunoprecipitation procedures are necessary to visualise the small amount of virus-specific polypeptides. The specificity of the immunoprecipitation was checked as follows. Immunoprecipitation of control injections without mRNA (Fig. 1), by addition of anti-AMV serum, gives rise predominantly to two polypeptides with molecular weights of 190,000 and 50,000, respectively. The latter aspecific polypeptide is also always found when the reticulocyte lysate is the assay

Fig. 3 Autoradiograph of the SDS gel electrophoresis pattern of products synthesised in reticulocyte cell-free incubations. Native 60S AMV RNA was incubated for 60 min at 30 °C in a reticulocyte cell-free system as described elsewhere¹⁰. The final incubation volume was 25 µl. 2 µl of the incubation mixture was analysed directly on the SDS slab gel and another 20 µl was immunoprecipitated¹⁶ and analysed. The Kodak X-ray film was exposed in contact with the dried gel for 2 d. *a*, Incubation in the reticulocyte cell-free system with lens 14S mRNA; *b*, control incubation without mRNA; *c*, incubation programmed with native 60S AMV-RNA; *d*, immunoprecipitate of an incubation with native 60S AMV RNA; *e* immunoprecipitate of polypeptides present in pulse labelled AMV-infected chick embryo fibroblasts.

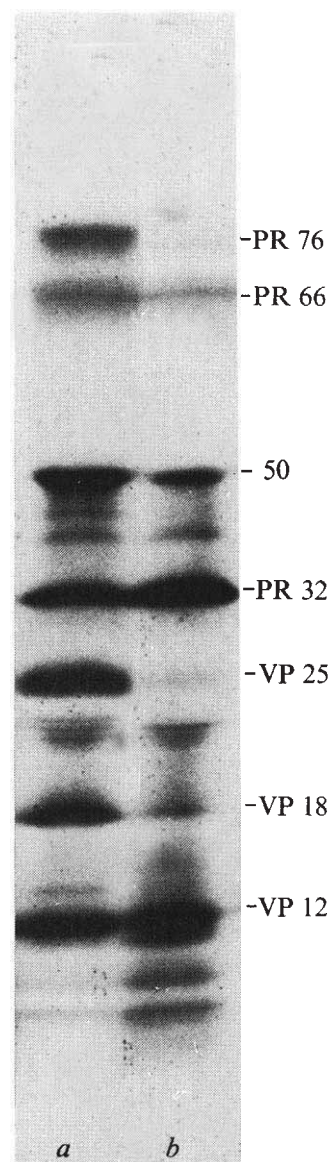
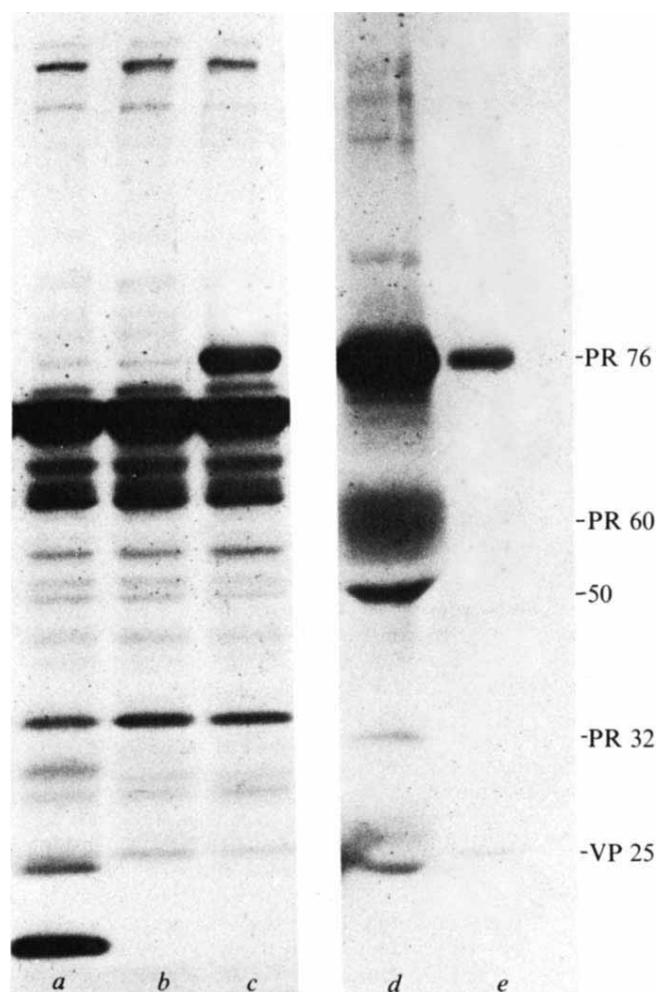


Fig. 4 Autoradiography of immunoprecipitated products synthesised in the reticulocyte cell-free incubation and analysed by SDS gel electrophoresis. The analysis of the virus-specific polypeptides synthesised in the reticulocyte cell-free system were as described in the legend to Fig. 3. The X-ray film was exposed in contact with the dried gel for 3 d. *a*, Immunoprecipitation of an incubation in the reticulocyte cell-free system with native 60S AMV RNA; *b*, immunoprecipitate of an incubation in the reticulocyte lysate with denatured 35S AMV RNA.

system. The same two polypeptides are detected as the only components after injection of AMV 60S RNA into the oocyte system followed by the addition of an antiserum directed against the non-related murine RLV (not shown).

From results obtained *in vivo* and *in vitro* we conclude that AMV RNA is translated into several precursor polypeptides which are cleaved into structural viral proteins, presumably by the same relatively unspecific enzymes present in chick embryo fibroblasts, oocytes and reticulocytes. We therefore believe that the oocyte system provides a useful tool for the study of the regulation processes involved in the expression of different viral functions *in vivo*.

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and Dr G. de Boer for leukaemia-free chick embryos. This work was supported in part by the Netherlands Foundation for Chemical Research and the Netherlands Organisation for Pure Research.

Note added in proof: We have also succeeded in translating Rauscher leukaemia virus RNA in oocytes. The precursor of the gs antigens pr65 and the processed polypeptides p30 and p15 have been detected.

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Homology of myosin DTNB light chain with alkali light chains, troponin C and parvalbumin

THE interaction of actin and myosin which occurs during muscle contraction is regulated by changes in intracellular Ca^{2+} concentration. In vertebrates Ca^{2+} regulation is associated with troponin and tropomyosin in the thin filaments, and the myosin ATPase activity in the presence of pure actin is not Ca^{2+} sensitive. Most invertebrates possess a myosin-linked (thick filament) control system, usually in conjunction with actin-linked (thin filament) control¹. Although the mechanism of thin filament regulation is fairly well characterised, that of thick filament control remains somewhat obscure (for reviews, see refs 2 and 3). This letter presents evidence for a structural and evolutionary relationship between proteins which have central roles in the two types of regulatory systems.

Myosin is composed of two heavy chains and four light chains. In rabbit white skeletal muscle, there are two structurally distinct⁴ classes of light chains, most commonly known as alkali light chains (ALC) and DTNB light chains (DLC)⁵. ALCs have long been thought to have an essential function in the ATPase activity of myosin, but until recently no function could be demonstrated for DLCs. Although there is some evidence that Ca^{2+} exerts a direct effect on rabbit skeletal myosin, and that DLCs may somehow be involved in regulation of actin-myosin interaction⁶⁻¹¹, as yet there has been no direct demonstration of myosin-linked Ca^{2+} control. DLCs, either isolated or as part of the whole myosin molecule, bind one Ca^{2+} per mol^{6-9,10,12} but Mg^{2+} competes strongly and so significant amounts of Ca^{2+} may not be bound in physiological conditions¹². In scallops (which

seem to lack troponin) specific light chains (the EDTA light chains, or ELCs) can be partially dissociated from the myosin, causing a loss of Ca^{2+} sensitivity which is restored on recombination^{13,14}. Even though DLCs may not function as regulatory proteins in rabbit skeletal myosin, they can substitute for scallop ELC in binding and restoring Ca^{2+} sensitivity to desensitised scallop myosin¹⁵. ELCs also bind to rabbit myosin from which DLC have been partially removed, but this hybrid is not Ca^{2+} sensitive. It thus seems that both scallop and rabbit myosins contain 'regulatory' light chains, and that lack of Ca^{2+} sensitivity in rabbit myosin is due to mutations in the heavy chains³. The amino acid sequence of rabbit skeletal DLC has been determined, using standard procedures previously applied to other muscle proteins¹⁶⁻¹⁸; experimental details will be published elsewhere. Comparison of the DLC sequence (Fig. 1) with the partially completed sequence of ELC (R. Jakes and J. Kendrick-Jones, unpublished) further confirms that the two proteins are closely related.

Previous comparative sequence studies^{16,19}, subsequently confirmed by more detailed analyses²⁰⁻²⁵, have suggested that the following rabbit skeletal muscle proteins are homologous (that is, they are descended from a common ancestor) and similar in three-dimensional structure: troponin C (TnC), the Ca^{2+} -binding regulatory protein of the thin filaments; ALCs, which do not bind Ca^{2+} in physiological conditions (J. Kendrick-Jones, personal communication); and Ca^{2+} -binding parvalbumin (CBP), a soluble protein of unknown function. By analogy with a carp CBP of known three-dimensional structure²⁶, the structure of TnC seems to consist of four very similar regions (I, II, III, IV), each of which contains a 12-residue Ca^{2+} -binding segment stabilised by short α -helices on either side. ALCs probably have a similar structure, although mutations in the four Ca^{2+} -binding segments have caused the loss in ability to bind in physiological conditions. The common ancestor of TnC, ALC and CBP was formed after two successive gene duplications produced a protein four times the length of an ancestral protein with a single Ca^{2+} -binding site. TnC is most closely related to the ancestor, since it has the strongest internal sequence repeats. CBP evolved by incomplete copying of the gene for the TnC-like precursor, causing deletion of region I and loss of Ca^{2+} binding in region II.

Analysis of the DLC sequence shows that it is also related to TnC. The sequences of rabbit skeletal muscle DLC, ALC (ref. 27), TnC (ref. 19) and CBP (ref. 28) are shown in Fig. 1 aligned with a uniform residue numbering system²³ based on the predicted structure of TnC. DLC are about equally similar to either TnC or ALC (an average of one of four residues are identical), less similar to CBP (about one of six identical residues). Of the two classes of light chains, ALC are slightly more similar to TnC. The phosphoserine-containing peptide isolated from DLC by Perrie *et al.*²⁹ is located near the N-terminus (residues NA8-NA18). As is the case with ALC, four homologous regions can be recognised in DLC, but the internal sequence repeats are very weak. Following the procedure used to predict the four Ca^{2+} -binding sites in TnC (ref. 16), the single Ca^{2+} -binding site of DLC is probably located in region I (residues abl-B3). The pattern of hydrophobic residues predicted to form the internal core of TnC is highly conserved in both DLC and ALC, suggesting that all these proteins have similar three-dimensional structures.

The finding that muscle cells contain a homologous group of structurally related proteins with seemingly diverse functions raises a number of important questions which may be answered by further comparative sequence studies. What are the structural alterations that caused the two classes of myosin light chains to evolve different functions? Since TnC and DLC are related what further similarities exist between the two types of muscle Ca^{2+} -regulatory

	NA1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
TnC DLC ALC	X-pro	lys	lys	ala	lys	arg	arg	ala	ala	ala	Ac-asp glu	thr gly	gln gly	gln ser	ala ser	glu asn	ala val	arg phe	SER SER	tyr met Ac-ser	leu PHE PHE	SER SER	
	23	24	25	A1	2	3	4 *	5	6	7	8 *	9	10	11	abl	2	3	4	5	6	7	8 *	9
TnC DLC ALC	glu gln ala	glu thr asp	met GLN GLN	ILE ILE ILE	ALA gln ALA	GLU GLU GLU	PHE PHE PHE	LYS LYS LYS	ala GLU GLU	ALA ALA ALA	PHE PHE PHE	asp thr leu	met val leu	phe ile tyr	ASP ASP ASP	ala gln arg	asp asn thr	GLY arg GLY	gly asn asp	GLY GLY ser	asp ile lys	ILE ILE ILE	ser asp thr
	B1	2	3	4 *	5	6	7 *	8 *	9	10 *	11 *	bcl	2 *	*	3	4 *	5 *	C1	2	3	4	5 *	6
TnC DLC ALC CBP	val lys leu	lys glu ser	glu asp gln	LEU LEU val	GLY arg GLY	thr ASP ASP	VAL thr VAL	met phe leu	ARG ala ARG	met ALA ALA	LEU MET LEU	GLY GLY GLY	gln arg thr glu	— leu —	thr ASN ASN	PRO val PRO	THR lys THR	lys glu asn ala	GLU GLU ala	GLU ASP GLU	LEU LEU val	ASP ASP LYS	ALA ALA LYS
	7 *	8 *		9	10	11	cdl	2	3	4	5	6	7		8 *	9	D1	2	3	4 *	5	6	7 *
TnC DLC ALC CBP	ile met val ala	ILE — leu ILE	— — gly —	glu — asn gly	glu — pro ala	val — ser phe	ASP — ASP —	GLU lys GLU ala	asp gln ala	gly ala met ALA	SER SER asn glu	GLY GLY ala ser	thr pro lys —	— lys —	ILE ILE ILE phe	ASP asn glu ASP	PHE PHE PHE his	GLU thr GLU lys	glu val gln lys	PHE PHE PHE phe	LEU LEU LEU phe	val thr pro gln	MET MET MET MET
	8 *	9	10	11	12	del	2	3	4	5	6	7	8	E1	2	3	4 *	5	6	7	8 *	9	10
TnC DLC ALC CBP	MET MET leu val	val phe glu —	arg gly ala —	gln glu ile —	met — — —	LYS LYS ser —	glu leu asn —	asp lys asn gly	ala LYS LYS leu	LYS gly asn LYS	gly ala gln lys	LYS asn gly LYS	SER pro thr SER	GLU GLU tyr thr	GLU asp GLU GLU	glu val ASP ASP	leu ile phe val	ala thr val lys	GLU gly GLU lys	cys ala gly val	PHE PHE leu PHE	ARG lys ARG his	ILE VAL VAL ILE
	11 *	eff	2	3	4	5	6	7	8 *	9	F1	2	3	4 *	5	6	7 *	8 *	9	10	11 *		
TnC DLC ALC CBP	PHE LEU PHE LEU	ASP ASP ASP ASP	arg pro LYS LYS	asn GLU GLU asp	ala GLY GLY lys	ASP lys ASP ser	GLY GLY thr GLY	tyr thr val phe	ILE ILE gly ILE	asp lys met glu	ala lys gly glu	GLU gln ala GLU	GLU phe GLU GLU	LEU LEU LEU LEU	ala glu arg gly	GLU GLU his phe	ILE leu val ILE	phe LEU LEU LEU	arg thr ala lys	ala THR THR gly	ser gln leu phe	— — — ser	— — — pro
		fgl	2	3	4 *	5	G1	2	3	4	5	6	7 *	8 *	9	10	11	ghl	2	3	4	5	6
TnC DLC ALC CBP	— — — asp	GLY cys GLY ala	GLU asp GLU arg	his arg lys asp	val phe met leu	thr SER lys SER	asp gln glu val	GLU GLU GLU lys	GLU GLU GLU GLU	ILE ILE val thr	GLU LYS GLU LYS	ser asn ala thr	LEU met LEU LEU	MET trp MET MET	lys ALA — ALA	asp ALA ALA ALA	GLY phe GLY GLY	ASP pro gln ASP	LYS pro glu LYS	asn ASP ASP ASP	asn val ser gly	ASP gly asn ASP	GLY GLY GLY GLY
	7	8 *	9	H1	2	3	4 *	5	6	7 *	8 *	9	10	11	12								
TnC DLC ALC CBP	arg asn cys lys	ILE val ILE ILE	ASP asn gly	phe TYR TYR ala	ASP lys glu ASP	GLU asn ala GLU	PHE ile PHE PHE	leu cys val ser	LYS tyr LYS thr	met val his leu	met ILE ILE val	glu thr met ser	gly his ser glu	val gly ile- ser-	gln- asp OH OH	OH ala	lys	asp	glu	gln-	OH		

Fig. 1 Alignment of the complete amino acid sequences of rabbit white skeletal muscle troponin C (TnC), myosin DTNB light chain (DLC), myosin alkali light chain (ALC) and Ca²⁺-binding parvalbumin (CBP). Amino acid residues are numbered in accordance with the predicted three-dimensional structure of TnC: helices are denoted A to H, from the amino to the carboxyl termini; interhelical loops are called ab, bc, and so on; residues preceding helix A are designated NA. Residues identical in two or more proteins are in capital letters, and those presumed to be involved in Ca²⁺ binding are underlined. Asterisks indicate residues predicted to form the hydrophobic core of TnC.

systems? What differences cause TnC to bind to troponin I and troponin T in the thin filaments, whereas ALC and DLC bind to myosin heavy chains in the thick filaments? These questions bring to mind Laki's proposal³⁰, that myosin could be considered as a complex of actin and tropomyosin (troponin had not then been discovered).

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Erratum

In the article "Entropy production in black holes" by W. Kundt (*Nature*, **259**, 30; 1976) the second paragraph should read:

To begin with, it must be remembered that in 'normal' conditions, matter has an entropy per particle $s \sim 1$ ($s = SN^{-1} k^{-1}$ where N = particle number): s varies between $\sim T/T_F \lesssim 1$ (for temperatures T below the Fermi temperature T_F) and $\lesssim 90$ (for dispersed hydrogen of critical cosmological density at $\gtrsim 10^8$ K); $s \sim 4$ for an extreme relativistic ideal gas . . . S_{bh} is $10^{19} (M/M_\odot)$ times larger than S at formation.

The second s^a in line 6 and s^a in line 15 of paragraph 4 should read s^a ; the divergence of s^a .

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reviews

GORDON PASK has always been something of an enigma on the educational and psychological scene. Operation from a private research company, although common in the States, is still a source of puzzlement in Britain. For many years Pask remained outside any comprehensible 'establishment' with a reputation and stature that developed, and was recognised, far more outside his own country than within it. His diversity of interests, from deep epistemological problems and system theory to practical training and the construction of elaborate electromechanical automata exhibiting 'learning' or inducing training, have also made him difficult to place. His writings* make few concessions to the reader, rarely simplifying what Pask already regards as an oversimplified view of a very complex world, and expressing it in a wide vocabulary appropriate to the richness and breadth of his conception of that world. Although one may admire an attempt to face up to a major part of the world of human acquisition of knowledge in its own terms, one may, however, also question its timeliness with regard to the intellectual and computational tools currently available to us.

Although there are few nowadays who would question the depth or logical consistency of Pask's approach, there may be many who will question its wisdom. The history of science is full of premature attempts to consolidate major areas of knowledge, before the correct viewpoint, reasonable objectives, or appropriate logicomathematical tools have been established. Pask adopts the all-embracing methodologies of Wiener's cybernetics and Bertalanffy's general systems theory and applies them to the highest levels of human behaviour and to practical problems of teaching and training. To what extent is he successful? I cannot give a clear-cut answer to this question. Neither will I pretend that the answer lies in this one book. What is clear, however, from grappling with the material in this book, both in its original form (as published papers) and in its present integrated form, is that the questions of 'success' cannot be answered negatively.

**Conversation, Cognition and Learning: A Cybernetic Theory and Methodology*. By Gordon Pask. Pp. xi+570. (Elsevier: Amsterdam, Oxford and New York, 1975.) Dfl.96; \$36.95.

Common sense and the computer

"Pask has put much effort into the development of actual teaching systems for major subject areas . . . and the practical experience and exemplary material developed is used to give life and content to the abstract, theoretical concepts put forward . . . No-one who takes the trouble to immerse himself in the work can fail to gain from the experience."



Gordon Pask

Pask has put much effort into the development of actual teaching systems for major subject areas, such as probability theory and statistics, and the practical experience and exemplary material developed is used to give life and content to the abstract, theoretical concepts put forward. There is a healthy atmosphere of operationalism throughout the work that convinces one that the cybernetic terminology is significant and applicable. No-one who takes the trouble to immerse himself in the work can fail to gain from the experience—Pask teases at basic problems from many directions at many levels and the discussion throughout is highly stimulating.

Although psychologists and system theorists will find the book of interest, its most immediate contribution is probably to studies of computer-based instruction (CAI). Pask's own use of the computer is slight—indeed, perhaps much of the insight he gives is because his structures are natural ones not distorted to fit a standard 'flow-chart' or use the keyboard of a teleprinter! His discussion of the learning and training strategies appropriate to the 'serialist' and 'holist' thinking on the one hand, and his demonstration of the complex entailment structures of comparatively simple subject areas on the other, illu-

minate major problems of CAI (and other forms of programmed instruction).

There is a paradox in our ability to develop teaching systems that work quite well for a wide range of individuals, whereas we know that there is a massive variation in student's tutorial requirements. Pask shows how performance feedback in adaptive teaching systems can tailor tuition to the individual without requiring detailed identification of his traits, provided broad differences in learning strategies can be recognised and the structure of the subject area has been thoroughly analysed. To the practising teacher such insights may seem common sense but no-one has so far successfully incorporated the common sense of the human teacher in a computer program. It is fundamental work, such as that described by Pask, that is essential if this is to become a reasonable objective.

The evaluation of Pask's work must be left to posterity—one hopes that this book will have several successors, developing and elucidating his theories and techniques. The present work must be welcomed as an opportunity to analyse, evaluate and utilise a substantial body of original and stimulating psychological and educational research.

B. R. Gaines

Steroid biochemistry

Biochemistry of Steroid Hormones. Edited by H. J. Makin. Pp. x+358. (Blackwell Scientific: Oxford and London, 1975.) £16.50.

THE stated aims of this book are to provide a simple and concise source of information on steroid biochemistry for preclinical and BSc students, and an introduction to the subject for postgraduates. In seven chapters, several experts deal with the subject from structure and nomenclature of steroids through biosynthesis of cholesterol and steroid hormones, to control of steroidogenesis and catabolism and excretion. Two useful chapters on methods of estimation of steroids follow. The remaining five chapters deal with the physiology and pathology of steroid endocrine systems, and include chapters on the endocrinology of the menstrual cycle and pregnancy, inborn errors of corticosteroid biosynthesis and molecular mechanisms of steroid hormone action.

Generally speaking, the book fulfils its aims very well, in that it provides a comprehensive and reasonably up-to-date account of this enormous and frequently daunting subject, well illustrated by means of key experimental data, without burdening the reader with an indigestible welter of detail. Perhaps the most forbidding aspect of steroid biochemistry to a newcomer is the apparent complexity and variety of structure and interminable pathways of biosynthesis and catabolism. These problems, although not hedged, are dealt with in a sympathetic and matter-of-fact fashion. The difficulty is acknowledged and the aim is to establish and reveal the order and logic behind the structure and metabolism of these compounds. These aims are started in the first

paragraph of the first chapter, by Professor Kellie, which in itself should go a long way to allaying the fears of the reader.

As always, criticisms can be made of details, and these are inevitably subjective to some extent. For example, in Fig. 4.10, the major biosynthetic route to 11β -hydroxy-4-androstenedione in the adrenal cortex is presented as proceeding from cortisol. Although this may be a conversion product of secreted cortisol, the major route to its biosynthesis in the adrenal gland itself is surely from C-19 precursors. Also, at the end of the book there is a pull-out scheme of steroid biosynthesis designed to assist readers of individual chapters, which may be too scanty in detail to achieve its aim.

These are, however, inevitable and minor criticisms of what is a very pleasing and useful book, which should prove of great benefit to student and teacher alike, although the price, at £16.50, might be somewhat daunting to both.

Evan Simpson

Tracking down nuclear particles

Nuclear Tracks in Solids: Principles and Applications. By Robert L. Fleischer, P. Buford Price and Robert Walker. Pp. xix+605. (University of California: Berkeley, Los Angeles and London, November 1975.) £20.50.

FOR scientists in the UK the field of nuclear tracks in solids must surely be a frustrating example of the one that got away! Work at Harwell in the late 1950s showed that damage trails in mica produced by fission fragments could be made visible in the electron microscope. This observation was sufficient to initiate a tremendously fruit-

ful collaboration between the American authors of this book, who, within a few years, demonstrated that such damage trails could be enlarged by etching so as to be visible optically, showed that a whole range of materials other than mica would store similar tracks and applied the technique to a great variety of fields. They have now crowned their achievement with an excellent book which treats in detail many aspects of nuclear tracks in solids and conveys a vivid impression of how important this technique has become.

The book is divided into three parts. The first deals with formation of particle tracks, principles of track etching and methods by which particles may be identified by the damage trails they leave behind them. The second discusses the application of track techniques in the Earth and space sciences through fission track dating of rocks, glasses, and so on, the study of the present flux of energetic particles in space, and of stored records of past fluxes of such particles in meteorites and lunar rocks. The final part of the book deals in rather less detail with applications of the technique to nuclear physics, element mapping in rocks and other materials, radiation dosimetry and many other diverse areas in science and technology.

The book is very well written and beautifully illustrated with photographs and diagrams which considerably aid understanding of the text. The mix of information given and information only referred to in other sources is very well judged and the many references, given as appendices at the end of each chapter, have each been given a title which much increases their usefulness.

This is an excellent book worth every penny of its purchase price. It must rapidly become a standard work indispensable to those most directly involved and of great interest to many others in allied fields.

R. N. F. Walker
P. H. Fowler

Analysing metal compounds

Mass Spectrometry of Metal Compounds. Edited by J. Charalambous. Pp. 297. (Butterworth: London and Boston, Massachusetts, 1975.) n.p.

THE first four chapters of the book deal with the theory of mass spectrometry, instrumentation and techniques. The next five chapters are concerned with specific groups of metal compounds, whereas the last chapter discusses analytical aspects

of the mass spectrometry of metal chelates. Finally, an appendix gives a table of nuclidic masses and abundances of the naturally occurring isotopes. There are over 400 references, many of which occur in the literature of the past decade or so, illustrating the rapid growth of inorganic mass spectrometry during the period.

There are a few minor criticisms of the book. In the first place it is surprising that in a comprehensive review of the energetics of molecular ionisation, no mention is made of the electron monochromator of Lossing, which provides the most accurate

method of determining ionisation and appearance potentials by mass spectrometry. Again the classical equation $k(E) = v[(E - E_0)/E]^{n-1}$ is used in discussing fragmentation kinetics, in spite of the fact that it is known to be an extremely bad approximation. On the positive side, references are made to studies of negative ions, which are becoming increasingly important.

Within the confines of production from typescript, the book is well produced. It should become an essential addition to the library of anyone working in the fields covered.

Allan Maccoll

Describing chemical methods

Methodicum Chemicum: A Critical Survey of Proven Methods and their Application in Chemistry, Natural Science, and Medicine. Edited by Friedhelm Korte. Volume 1: Analytical Methods. Part A: Purification, Wet Processes, Determination of Structure. Pp. x+1-628. Part B: Micromethods, Biological Methods, Quality Control, Automatisations. Pp. x+629-1218. (Academic: New York and London; Georg Thieme: Stuttgart, 1975.) \$98; £47.05 the two parts.

VOLUMES 1A and 1B comprise the start of an eleven-volume series which will give "a short critical description of chemical methods applied in scientific research and practice", the objective being "to provide the chemist and the scientist working in related areas of chemistry with a rapid and reliable source of information on the method applicable to his specific problem."

The subtitles of volumes 1A and 1B "Analytical Methods", is somewhat misleading, especially for 1A, as it is used in the sense of integral identification of a molecular entity and not in

that of conventional chemical analysis; moreover the emphasis is strongly biased towards organic and biological chemistry. With these reservations the editors and about 100 contributors, almost entirely European, have managed to compress into 1,200 pages a vast quantity of well presented information. The methods discussed, some of the less well known purposely given in greater detail than others, range from simple chemical operations to highly sophisticated instrumental techniques. The individual chapters provide a guidance to workers in the selection of methods appropriate to the problem being tackled and at the same time give sufficient theoretical background to enable the user to judge whether or not he can use the selected procedure himself or must employ the services of a specialist in the chosen field.

Part A deals with small-molecule separation, identification and structural analysis. Chemical and other methods are described for functional groups, but specialised techniques such as X-ray diffraction (40 pages) and various spectroscopic methods (200 pages) are dealt with in greater detail.

Part B first discusses some special physical methods covering such diverse topics as the determination of molecu-

lar weights, electron donor-acceptor complexes and electric dipole moments. Thereafter it deals with specific chemical, instrumental or biochemical techniques for the determination of both trace elements and organic constituents in a wide variety of organic samples including foods, fats, water, fuel and pharmaceuticals.

The work will provide a source of condensed information on newer techniques and, although it will not replace specialised monographs, adequate references up to 1969, sometimes up to 1972, are supplied for readers desiring more detailed information. The 53-page index, however, has omissions, no reference for example being given to the section on the use of flame photometry and atomic absorption for the analysis of fuel oils on p.955. The high cost of £48.05 will be beyond most private buyers but is justified by the amount of work that has been carried out by the authors and editors. The quality of production is excellent although somewhat marred by spelling errors and by the use of the symbol J for iodine in several places. If the following volumes maintain the quality of Volumes 1A and 1B, the series will become a reference work of major importance for all multidiscipline establishments.

R. O. Scott

Molecular complexes

Molecular Association. Volume 1. Edited by R. Foster. Pp. xiv+365. (Academic: London, New York and San Francisco, August 1975.) £11.80; \$31.25.

THIS book contains four articles dealing with various aspects of electron-donor-acceptor (EDA) molecular complexes or charge-transfer (CT) complexes as they are commonly, but imprecisely, known, and with other molecular complexes. Z. G. Soos (Princeton) and D. J. Klein (Austin) give a detailed account (109pp, 340 refs) of charge transfer in solid-state complexes. This includes a phenomenological theory, a classification of π -molecular CT crystals, one-dimensional Hubbard models, magnetic properties of CT crystals, and the computation of model parameters. N. Kulevsky (Grand Forks) describes the dielectric properties of molecular complexes in solution, with particular reference to halogen complexes, complexes of π^* -acceptors, and halogenated alkane interactions (50 pp, 103 refs). K. M. C. Davis (Leicester) discusses solvent effects on 'CT complexes', including the position and intensity of the CT absorption and

fluorescence bands, charge-transfer-to-solvent (CTTS) spectra, vibrational spectra of complexes, nuclear magnetic resonance shifts, thermodynamic parameters, and complex-solvent interactions (63 pp, 334 refs). R. S. Davidson (Leicester) provides a comprehensive survey of photochemical reactions involving 'CT complexes' (120 pp, 449 refs). These are classified into reactions involving excited 'CT complexes', excimers (intermolecular and intramolecular) and exciplexes (subdivided into those with and without spectroscopic evidence).

There have been several recent books on molecular complexes, three of them written (*Organic Charge-Transfer Complexes*, Academic: London and New York, 1969) or edited (*Molecular Complexes*, 1 and 2, Elek Science, London; Crane Russak, New York, 1973 and 1974) by R. Foster, who has skilfully chosen the authors and contents of the present volume to cover topics he has not previously considered. It is doubtful whether there are sufficient gaps remaining in the literature to justify the initiation of a new series of volumes on molecular complexes under a different title selected "for bibliographical convenience." *Molecular Complexes*, Vol. 3, would have been a more accurate and convenient title for the present book. J. B. Birks

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obituary

Mr Ernest Gold, CB, DSO, OBE and FRS died on January 30 at the age of 94. He was most famous for his early work on the stratosphere. In 1907 he had become the Schuster Reader in dynamic meteorology at Cambridge, and a year later he published his paper explaining the existence of the stratosphere. That a region sharply delineated by a boundary (the tropopause), where the tendency for the temperature of the air to decrease with height abruptly stops and the temperature becomes constant, seemed paradoxical. Gold's explanation of this effect, taking into account the carbon dioxide and water vapour contained in the atmosphere is still accepted, though it is a measure of the complexity of calculations in weather science that no quantitative solutions for the effect have yet been achieved. In 1910 he left Cambridge for the Met. Office, building up its stature and importance. With the advent of aviation, the need for international weather forecasting became apparent, and it was Gold's achievement to mastermind the setting up of this service, as president of the International Commission for Synoptic Weather Information, a position he held for 28 years.

Clair L. Stong, conductor of the 'Amateur Scientist' column in 'Scientific American' since 1957 died on Tuesday, December 9, of cancer. He was born in Douds, Iowa, and was 73 years old.

Mr. Stong, known to everyone as 'Red', first became known as an aviator. While studying electrical engineering at the University of Minnesota and the Crane Institute, he earned his keep as a stunt pilot: for a while leading his own flying circus and performing at prairie county fairs. He joined the Western Electric Co. in 1926, working in various capacities until retiring in 1962 to devote himself full-time to the Scientific American.

In his Amateur Scientist column, he opened up the 'black boxes' of modern science. He showed his readers how to build in their kitchens and garages such formidable instruments as atomic particle accelerators and cloud chambers and scintillator counters to go with them, quartz-crystal clocks, lasers, ruling engines, high-altitude rockets and both digital and analogue computers. Experiments specified in his column ranged over the frontiers of science from molecular biology (how to make amino acids) to experimental

psychology (colour vision in pigeons) to oceanography (how to make a hydrophone) to cosmology (eclipses of stars by the mountains of the moon). His readers could rely on his instructions because he had himself built nearly all the instruments, and performed nearly all the experiments he described.

His column on hang gliding in 1973 is credited with helping to make that daring sport a national pastime. An anthology of his pieces published in 1960 has been translated into a dozen languages, and with James E. Hammesfahr he wrote 'Creative Glassblowing' in 1968, a handbook for amateurs now widely consulted by laboratory technicians.

That his experiments were performed by his readers was also evident for many years from high school science fairs across the country; the American Association for the Advancement of Science at its meeting in Boston this February has scheduled a session on the role of Mr. Stong's column in stimulating careers in the sciences.

Mr. Stong will be missed by his wife, his two children, and all his readers. The Amateur Scientist column is being discontinued. **Gerard Piel**

announcements

International meetings

March 11, **New Particles and New Quantum Numbers**, a discussion meeting, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1 5AG, UK).

April 8-10, **Natural Gas Processing and Utilisation**, Dublin (Natural Gas Conference, Institution of Chemical Engineers, P.O. Box 770, Upper Merion Street, Dublin 2, Ireland).

April 12-13, **Drug Action at the Molecular Level**, organised by the Biological Council Coordinating Committee for Symposia on Drug Action, London (Miss G. Blunt, Administrative Secretary, c/o Department of Pharmacology, University College, Gower Street, London WC1E 6BT, UK).

April 12-15, **Unsteady flow in many channels**, Newcastle-upon-Tyne (The Organising Secretary, U.F.O.C. Symposium, BHRA Fluid Engineering,

Cranfield, Bedford MK43 0AJ, UK).

April 12-16, **Spring Annual Meeting**, Washington D.C. (Meetings, AGU, 1909 K Street, NW, Washington, D.C., 20006).

April 15-17, **Social and Economical Significance of Animal Production Policy of EEC and The World Strategy for Zootechnical Production**, Milan, organised by the Societa' Italiana per il Progresso della Zootecnica, Milan (Professor T. Bonadonna, Milano, Via Comelico 3, Italy).

April 20-23, **Third European Meeting on Cybernetics and Systems Research**, Vienna (Secretariat of the Austrian Society for Cybernetics Studies, Schottengasse 3, A-1010 Wien, Austria).

April 20-24, **Models and Numerical Methods Applied to the Studies of Surfaces and of Adsorbates on Surfaces**, CECAM, Paris (Professor M. Simonetta, Istituto di Chimica Fisica, University of Milan, Milan, Italy).

April 22, **European Solar Houses**,

London (The Secretary, UK-ISES, The Royal Institution, 21 Albemarle Street, London W1X 4BS, UK).

April 23-24, **Historical Biogeography, Plate Tectonics and the Changing Environment**, Corvallis, Oregon (A. J. Boucot, Department of Geology, Oregon State University, Corvallis, Oregon 97331).

April 24-30, **Radiation Protection as an Example of Action Against Modern Hazards**, Paris (Mr Gilbert Bresson, General Secretary IRPA Fourth International Congress, B.P.33,92260-Fontenay-aux-roses, France).

April 25-28, **The 22nd Annual Meeting**, Philadelphia (Institute of Environmental Sciences, Betty Peterson, Executive Director, 940 East Northwest Highway, Mount Prospect, Illinois 60056).

April 27-29, **New Materials and Processing in the Textile Industry**, Manchester (Mr J. K. Jackson, Shirley Institute, Manchester M20 8RX, UK).